

BULLETIN No. 437.

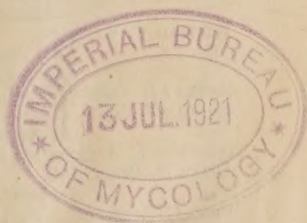
JULY, 1917.

New York Agricultural Experiment Station.

GENEVA, N. Y.

NECK-ROT DISEASE OF ONIONS. X

M. T. MUNN.



PUBLISHED BY THE DEPARTMENT OF AGRICULTURE.

CONTENTS.

	PAGE.
Summary.....	365
Introduction.....	367
History of present investigation.....	367
Economic importance of the onion.....	367
New York onion districts.....	368
Michigan onion districts.....	368
The disease.....	369
Name.....	369
Plants affected.....	369
Distribution.....	369
Economic importance.....	370
Estimate of losses in Michigan and New York.....	371
Nature of the losses: To the growing crop and seed crop.....	372
Signs of the disease.....	373
On the leaves, on the necks and on the bulbs.....	373
On the roots.....	374
On the seed heads.....	375
Etiology.....	375
Infection of dry bulbs by dusting with dry spores.....	375
Infection of growing plants by means of dry spores.....	376
Infection of growing plants by means of spore suspensions.....	377
Effect of size of drop of suspension upon infection.....	379
Infection by placing spores between leaf sheath and neck.....	380
Infection thru incisions.....	380
Infection by means of sclerotia.....	381
Infection thru the flowers.....	381
Mycelium in necks of infected bulbs.....	382
Inoculation experiments with other plants.....	382
Summary and conclusions.....	383
The parasite.....	384
Morphology.....	384
Mycelium.....	384
Appressoria.....	385
Sclerotia.....	386
Conidia.....	389
Comparative morphology.....	390
The name of the fungus.....	391
Physiology.....	397
Sources of pure cultures.....	397
Growth on vegetable tissue.....	397
Growth on plant broths, etc.....	400
Growth on synthetic solutions.....	400
Growth on various agars.....	400
Thermal relations.....	401
Relation to environmental factors.....	404
Reaction to temperature.....	404
Reaction to light and to aeration.....	405
Reaction to desiccation and to moisture.....	406
Biochemical relations.....	407
Relation to acids and alkalis.....	407

	PAGE
Life history studies.....	409
Primary infection of bulbs in the field.....	409
Infection of bulbs at harvest time.....	409
Secondary infection of bulbs in storage.....	410
Overwintering and dissemination in the field.....	411
Dissemination in seed fields thru transplanting diseased bulbs.....	412
Occurrence of the fungus spores on onion seed.....	412
Relation of parasite to host.....	412
Historical review.....	413
Pathological histology.....	415
Cell walls of the host.....	416
Production of oxalic acid by the fungus.....	417
Oxalate crystals in media.....	418
Oxalic acid in the diseased tissues.....	419
Oxalates in the diseased tissues.....	419
Titration of liquid media.....	420
Acidity of sound and diseased onions.....	420
Action of oxalic acid solutions upon plant tissue.....	421
Production of an enzym by the fungus.....	423
Action of extract from old mycelium.....	423
Action of droplets exuded from upper surface of cultures.....	425
Action of extract from diseased bulbs.....	426
Action of the extract from young mycelium.....	427
Effect on tissue strips.....	430
Injection of the extract.....	433
Action of the extract upon cotton fibers.....	433
Physical and chemical properties of the extract.....	433
Effect of temperature upon the activity of the extract.....	433
Effect of age upon the action of the extract.....	433
Titration of the extract.....	434
Relation of chemical reaction to activity.....	434
An attempt to secure the enzym by precipitation.....	435
Effect of temperature upon the dissolved precipitate.....	436
Reprecipitation of the enzym.....	436
Precipitation of the enzym from old mycelium.....	437
Conclusions.....	438
Action of the fungus on cellulose.....	438
Immunity relations: Resistence of varieties.....	440
Control.....	440
Factors which favor infection or amount of neck rot.....	440
Maturity and degree of curing.....	440
Fertilizers.....	440
Soil types.....	441
Air drainage.....	441
Storage conditions.....	441
Storage experiments.....	443
Study of remedial measures.....	444
Spraying.....	444
Fumigation.....	446
Methods of control.....	448
Bibliography.....	450

NECK-ROT DISEASE OF ONIONS.¹

M. T. MUNN.²

SUMMARY.

The neck-rot disease of onions apparently occurs in every onion-growing district in Michigan and New York and in every principal onion section in the United States. It causes heavy losses to stored onions and frequently to the growing crop and the seed crop. Evidence has been secured that the disease is destructive in Europe. The most striking signs of the disease are to be found in the storage houses where the causal fungus appears as masses of sclerotia at the necks of the onions. However, these may cover part, or all, of the individual bulbs. The smoke gray covering of fungous growth commonly recognized by onion growers as "gray mold" is another striking sign of the disease.

The cause of the disease as determined by numerous inoculation experiments is a fungus which has been described and given the name *Botrytis allii*. Infection of the bulbs in the field occurs thru the leaves and necks. The fungus passes down the necks and causes a neck rot in the field, or else it goes into the storage house in the form of mycelium in the necks and bulbs where the disease develops rapidly under favorable conditions. The bulbs may also become infected from the soil. The seed

¹ Also presented to the faculty of the Michigan Agricultural College in partial fulfilment of the requirements for the degree of Master of Science.

² Acknowledgments are due Mr. F. C. Stewart, Botanist of this Station, and to Drs. Ernst A. Bessey and G. H. Coons of the Michigan Agricultural College for valuable aid in many ways during this study. Thanks are also due Professor H. H. Whetzel of Cornell University who read the portion of the manuscript relating to nomenclature and who, because of his knowledge of the genus *Botrytis*, was able to make many valuable suggestions. A number of prominent onion growers have made this work possible by placing their onion storage houses and onion fields at the disposal of the writer for the purpose of experimentation and observation. Special mention should be given the names of Messrs. C. C. Mowris and Henry Greffrath of South Lima, N. Y., L. J. De Martinas of West Bloomfield, O. L. Humphrey of Honeoye Falls, and the J. B. Frey Co. of Rochester, N. Y. The onion growers of Michigan, also, responded liberally in many particulars. Messrs. E. F. Clark & Son of Wayland, Michigan, deserve many thanks.

heads become infected by wind-blown spores, causing a blasting of the flowers.

The fungus apparently produces appreciable amounts of oxalic acid. However, it was not possible to demonstrate that this acid produces the pathological changes in tissue observed when the fungus attacked the host. It was found that the fungus secretes the enzyme pectinase. This enzyme was precipitated and re-dissolved from the fungal extract. From a study of the action of the fungal extract upon plant tissue discs it was possible to explain all of the tissue changes taking place when the parasite attacks the host.

Factors favorable to infection and the occurrence of the neck rot were found to be as follows:—(1) Immaturity and imperfect curing of the bulbs; (2) the application of commercial fertilizers late in the season or in incorrect proportions; (3) the application of large quantities of stable manure before planting; (4) poor air drainage in the onion fields; (5) high humidity, high temperature and poor ventilation in the storage house.

Methods of control, so far as worked out, lie mainly along the line of field sanitation, care of the curing crop, and storing in properly constructed and regulated houses. Diseased bulbs should not be used as "mother bulbs" for producing the seed crop since they are an important source of infection to the seed heads, and later, to the growing crop in nearby fields. Piles of decaying and refuse onions should not be permitted near the onion fields or used for fertilizer thereon. Care should be exercised in handling the crop to prevent unnecessary bruising on the topping machine and while handling the stock to be stored. The crop should mature and die early. Remove all refuse tops, soil and screenings. Cure thoroly in the field or in the drying sheds. Store the crop in slatted crates in well constructed houses having ample facilities for ventilation and the maintenance of low temperature.

Fumigation of the stock with formaldehyde gas either just before or after storing has been found to be both ineffective against the fungus and impracticable in the average storage house. While spraying the growing crop with bordeaux mixture has given some promising results, it has not been tested sufficiently to warrant definite recommendations. Apparently, the spraying of onions has not proven popular with onion growers because of certain difficulties encountered.

INTRODUCTION.

HISTORY OF PRESENT INVESTIGATION.

The incident which, in part, initiated this investigation, was the receipt on February 24, 1913, of specimens of diseased onions from Mr. O. L. Humphrey, manager of the Upton Storage Company's houses at Honeoye Falls, N. Y. A letter accompanying the specimens stated that the onions in the storage house were decaying rapidly and that the loss would be complete unless measures were at once taken to stop the spread of the disease. At the suggestion of Mr. F. C. Stewart, Botanist of this Station, an investigation was started. Upon visiting this storage house 700 bushels of yellow onions grown upon muck land were found stored in a rather damp basement. All of the onions were badly diseased, nearly every bulb showing the presence of large masses of black sclerotia encircling the necks, with an occasional specimen showing a gray, dense, close-growing weft of a fungus readily recognized as a species of *Botrytis*. The loss was practically complete. A visit to other onion storage houses in central and western New York revealed the fact that considerable storage loss was taking place because of this onion disease. It was evident that we were dealing with a disease of great economic importance and one whose etiology was obscure and control measures unknown. Further investigations and studies, herewith reported, were made during the years 1913, 1914, 1915, and 1916 from the Geneva Station, and during the fall of 1915 and winter and spring of 1916 at the Michigan Agricultural College. In Michigan, as in New York, the onion growers and storage house owners and operators had invariably the same experiences to relate. Their crops were apparently well cured and sound when stored; but a short time after storage began, about October or later, the stock became badly rotted, the rot increasing directly as the storage season advanced. The stock was sorted, but unless the apparently sound onions were marketed immediately the crop soon became an almost complete loss to the owners. Unusual care in curing and storing the crop did not appear to prevent the disease.

ECONOMIC IMPORTANCE OF THE ONION.

The Thirteenth Census of the United States shows that 47,625 acres of onions, valued at \$6,709,047 were produced in 1909, the

onion holding third place among the truck crops of the United States. Every state is reported as producing some onions. Both as to acreage and value of onions produced, New York and Ohio were the leading states. In 1909 the largest onion acreage for any geographical division was reported from the East North Central States, including Ohio, Indiana, Illinois, Michigan and Wisconsin. The Middle Atlantic Division, including New York, New Jersey and Pennsylvania reported the second largest acreage. The writer feels confident that the onion acreage as reported by the census is low; at least this appears to be true for the states of Michigan and New York. According to Blair (1916) the area planted with onions in 1915 in the principal onion producing states was estimated by the Department of Agriculture to be 49,573 acres.

NEW YORK ONION DISTRICTS.

According to the Thirteenth Census of the United States, New York State in 1909 ranked second in onion production, having an acreage of 5,558 acres, valued at \$954,610. Only seven counties in the State are reported as producing no onions. Orange County leads in onion production having approximately 2900 acres in the vicinity of Goshen, Florida, and Pine Island. Wayne County is second with 700 acres located near Rose, while Madison County is third with 570 acres in the Canastota region devoted to the production of this crop. The other important onion growing regions are in the counties of Queens, Monroe, Erie, and at South Lima in Livingston County.

MICHIGAN ONION DISTRICTS.

In Michigan the largest onion sections are in Allegan and Kent Counties. The Allegan acreage comprises the districts at Plainwell, Gun Marsh, Martin, Shelbyville and Wayland. The Kent County district is located between Byron Center and Ross. The other important districts are at Big Swamp in Lenawee County, Grant in Newago County, Zealand Township, Hudsonville and Vriesland in Ottawa County, Mentha, Van Buren County, and at Manchester in Washtenaw County. In the Thirteenth Census of the United States, Michigan is reported as having in 1909 an onion acreage of 1,130 acres, the crop being valued at \$146,507.

THE DISEASE.

NAME.

There are a number of common names applied to this disease. The list comprises Mold, Gray Mold, Storage Rot, Rot, Onion Rot, Onion Bulb Rot, Dry Rot, Black-neck Rot, Dry Neck-rot, Stem Rot, and Neck Rot. Of these names the last two seem to be more descriptive of the disease. The writer prefers the name Neck Rot since it more accurately describes the appearance of the disease in storage, a form which the grower readily recognizes. Furthermore, the term stem cannot be used, botanically, for the parts of the host plant above the bulb usually attacked by the fungus. Accordingly, the name Neck Rot will be used in this account.

PLANTS AFFECTED.

The neck-rot disease caused by a parasitic *Botrytis* seems confined to *Allium cepa*, a broad species with many widely varying varieties. It has been found on perennial or tree onions, on seed onions, and growing or market crop onions. Schröter (1879) reported a *Botrytis* on *Allium ursinum* but gives no description whatever of the fungus. Frank (1896) stated that he also observed near Leipzig the *Botrytis* noted by Schröter.

DISTRIBUTION.

That the neck-rot disease of onions, or what appears to be this disease, is widely distributed is indicated by reports from nearly every onion growing region. Sorauer, as early as 1876, reported a *Botrytis* disease of onions from Germany, while a few years later Frank (1880) reported it from the same place. Bruck (1907) also mentioned this disease as being common in Europe. Voglino (1903) stated that he found what appears to be this disease in Italy and France in 1892. An onion disease caused by a *Botrytis* was noted in England in 1894 according to Massee. Halsted (1890) appears to have been the first in America to report a neck rot of onions due to a *Botrytis*. Clinton (1903) reported this disease from Connecticut; Jarvis (1909) also reported it from that state. The disease was recorded by Selby (1910) as being abundant in Ohio, while Jackson (1914) stated that it is a serious storage trouble of onions in Oregon. Humbert (1916) reported heavy storage losses from this disease upon white onions in Ohio districts.

In the fall of 1915 the writer noticed the neck-rot fungus upon some large white onions in crates in the Grand Trunk Railway freight house at Lansing, Michigan. These onions were consigned to a Lansing dealer and were shipped from Bermuda. Isolations in pure culture confirmed this observation. Again, in 1916 and 1917, the *Botrytis* neck-rot fungus was found upon, and isolated from, large white onions shipped from Bermuda and also from Texas.

In a letter from Dr. Johanna Westerdijk of Amsterdam, Holland, addressed to Professor H. H. Whetzel of Cornell University and dated January 4, 1917, an interesting reference is made to a disease of the onion. Dr. Westerdijk supplied Professor Whetzel with a culture of a *Botrytis* which she stated caused a severe disease of the leaves of onion. A critical study of transfers from the culture furnished to Professor Whetzel showed the fungus to be identical in every particular with the cultures of the onion *Botrytis* secured from various places in America. Dr. Westerdijk also stated that she had described the disease in a brief paper. Unfortunately, no further information regarding the disease could be obtained from this source at this time.

A report based upon the records¹ of the Plant Disease Survey (Bureau of Plant Industry) show that the *Botrytis* neck rot of onions has been reported by collaborators as being serious in the states of Connecticut, Illinois, Maryland, Massachusetts, Michigan, New York, Ohio, Oregon, Pennsylvania, Washington and Wisconsin.

ECONOMIC IMPORTANCE.

The margin of profit on storage onions is usually small, and unless a marked increase in price takes place a small amount of neck rot will offset the gain from storage. The neck-rot disease has practically prevented the growing of white onions in many onion sections in New York and Michigan. Figure 1, plate V, shows one of a number of white onions taken from an upland field early in the season of 1915. Their condition indicates the virulence of the fungus upon these tender, thin-skinned varieties.

¹ These data are from unpublished records of the U. S. Dept. Agr. Bur. Plant Indus., Plant-Disease Survey. They include reports from states where *Sclerotium cepivorum* is believed to be the cause of the disease, also *Botrytis cinerea*, *Botrytis vulgaris*, *Sclerotinia fuckeliana*, *Sclerotinia* sp., and *Sclerotinia* (bulb rot) are reported as causing what is believed to be this disease of onions.

A study was made of the losses due to the neck-rot disease occurring in the retail trade of grocery stores dealing in southern onions. With the flat white onions from Texas a loss of about two per ct. occurred, this increasing slightly as the spring season closed. Frequently, one finds a badly diseased specimen among the large, white bulbs sold as Spanish and Bermuda onions. These are usually sold by the pound and the losses are correspondingly greater. The records of the Plant Disease Survey as furnished by collaborators in the states mentioned in the preceding section show that the losses vary from 7 or 8 per ct. to as high as 50 per ct. of the crop. In two instances it is reported that the acreage devoted to onion growing has been materially decreased because of the ravages of this disease. The highest losses are plainly associated with seasonal conditions which are termed "poor seasons for onions." Neck rot is plainly one of the limiting factors in onion growing.

ESTIMATE OF LOSSES IN MICHIGAN AND NEW YORK

For these states as a whole it is difficult to obtain any accurate data covering the losses in the yellow and red varieties of onions. The estimates must be limited to individual storage houses. During the storage season of 1912-13 the losses in some poorly ventilated houses were almost total, while in some houses of the better class the average loss was approximately one crate out of every ten at the time of sorting before shipment. During the storage season of 1915-16, following an exceedingly unfavorable growing season for onions, the storage losses were enormous, being practically total in many storage houses. In Michigan, early in the winter of 1915, the loss from neck rot in several storage houses was averaging one crate out of every six sorted, while by January 4 the losses were slightly over 50 per ct. of the total stored crop. Some definite, accurate data furnished by one prominent onion grower are as follows: Owing to a poor onion year this particular grower harvested about 25 per ct. of a crop. This gave 2200 bushels which were put in storage. The neck rot appeared in a severe form in less than six weeks after the stock was placed in storage. Of the 2200 bushels stored, 1200 were dumped out on the fields. The owners were able to realize upon but 1000 bushels. Of the entire crop only two bushels of bulbs were fit to save for producing a seed crop.

NATURE OF THE LOSSES.

To the growing crop. — The most important factor to be considered in this connection is the effect of the disease on the keeping quality of the bulbs. At the time of sorting, the neck-rotted bulbs are included with those attacked by the smut fungus, onion maggots, and all sprouted bulbs. This material is classed as "shrinkage" by the onion grower. A careful analysis of this "shrinkage" into its component parts showed it to be about 60 per ct. neck rot in some instances. An examination of many of the sprouted bulbs showed that the neck-rot fungus may have been the initial or exciting cause of the sprouting in many cases, since some moisture is present after rotting takes place. In one storage house under observation the loss due to the neck-rot fungus, six weeks after the crop was placed in storage, was approximately one crate out of every five — a "shrinkage" of about 20 per ct. In this particular house the loss was nearly complete by January 30.

Where the fungus attacks the bulbs thru the roots and the necks in the field the loss is due to a decrease in tonnage per acre because of a failure of the affected bulbs to produce size. The small-sized onions are classed as "seconds" and "picklers" and command a much lower price than the "primes."

Severe attacks of neck rot may follow frost injury to the crop early in the fall. Frosted onions are usually immature and bring considerable moisture into the storage house.

To the seed crop. — It is impossible to obtain accurate data as to the losses occurring in the seed crop since it is difficult to separate two factors which cause a failure of the seed crop, viz.: (1) Excessive moisture, which often prevents a setting of the seed heads; and (2) attacks of the neck-rot fungus, which also cause a failure of the flower heads to produce seed. Apparently, the two factors are inseparable, i. e., an attack of the fungus follows a period of excessive moisture. There is a so-called diseased condition brought about by excessive drought at the time of seed formation which is known to the onion seed growers as "blast." This condition is to be differentiated from the blasting of the seed heads due to the neck-rot fungus.

A general failure of the onion seed crop in certain regions in Michigan in 1915, and in some sections of New York in 1913 is believed by the writer to be due to the attacks of this fungus. Clinton (1903,

1914) reported the *Botrytis* fungus as causing a yellowing of the leaves and a blasting of the onion flowers early in the season in Connecticut. Here it was reported as doing considerable injury to the flowers in moist seasons.

SIGNS OF THE DISEASE.

ON THE LEAVES.

Where infection has taken place on the leaves thru the germination of conidia one finds at first small white spots which later enlarge. The diseased spots are very frequently elongated in the same direction as the cells of the host plant. The spots may be surrounded by a yellowish area of tissue having a water-soaked appearance. This is to be distinguished from a so-called physiological disease of onion leaves known as White Tip. In this the tips of the leaves turn white, whereas the neck rot fungus causes a slight yellowing of the leaves. Sometimes infection may occur where the leaves are folded over or following severe injury by thrips. Shortly following infection, in any case, the tissue adjoining the point of infection takes on a water-soaked or boiled appearance, the green color of the leaf disappears and is replaced by a yellowish brown. Clinton (1903) reported that he found the fungus (an onion *Botrytis*) appearing as a parasite on the green leaves comparatively early in the season.

ON THE NECKS.¹

The disease occurs on the necks in the form of tufts of conidiophores bearing smoke-gray conidia (Plate V, figs. 2, 3). The fungus may be readily found upon the necks of the "scallions," "bull-necks" or "thicknecks" which are put in storage. On these the fungus readily shows itself because the bulbs are not dry or mature when they go into the storage house. From the neck and outer leaves the fungus works down into the bulbs killing the tissue of the host in advance of the mycelium.

ON THE BULBS.

The neck rot is usually first brought to the notice of the onion grower by the appearance of roundish black bodies, or sclerotia,

¹ The term neck as used in this paper is applied to that part of the onion plant just above the bulb, which resembles a stem, namely, the leaves which are enclosed in the sheath of the outermost leaf and extending well down to a point where the leaves become markedly thickened forming a bulb.

upon the onion bulbs in storage. The sclerotia are more frequently found at the necks of the bulbs occurring either singly or in the form of sclerotial crusts which may encircle the entire neck of the bulb (Plate VI, fig. 3). These sclerotial crusts at the necks may be pulled off rather easily, carrying with them the remains of the outer two or three bulb scales, or thickened bases of leaves, from which they are inseparable. These sclerotia may also be found upon the sides or bottoms of the bulbs, and often one finds a specimen with the entire side covered by a dense sclerotial crust (Plate VI, fig. 4).

Occasionally, a bulb will be found which shows the presence of the sclerotia between the scales. The bulb may be very soft, contain considerable liquid and give off an offensive odor. In such cases it is believed that soft-rot bacteria follow the invasion of the fungus as secondary rot producers. As a rule but very little moisture is formed following the attacks of the fungus. It is because of this fact that this disease has been called dry rot and dry neck rot by some.

Some bulbs, especially those from the dry locations in the storage houses, will show a smoke-gray covering of the conidia and conidiophores of the fungus in the region of the neck. This form of the fungus is very frequently found on the flat white onions shipped to the northern states from the southern onion growing regions. Other bulbs may show the presence of both the sclerotial and the conidial stage of the fungus at the same time. Upon cutting into the diseased bulbs one frequently finds the spaces between the scales filled with a mat of mycelium varying in color from a dirty white to a dark brown. On cutting open bulbs which show only a few tufts of conidiophores at the neck, the bulb scales are nearly always found to be softened and discolored in the region of the neck and may show a mat of mycelium present.

ON THE ROOTS.

The fungus has been found causing a serious root rot of the bulbs in the field. The condition brought about by the root rotting is very noticeable later when the bulbs are put in storage where the fungus appears in the form of either sclerotia or tufts of conidiophores at the bottom end of the bulb, and also by the fact that the bulbs are considerably smaller than the average run of stock. When the sclerotia are formed at the bottom end of the bulbs they are frequently in the form of a ring or crust surrounding the base of

the onion. Data obtained from storage inspections show that there was more root or bottom infection during the year 1915 than in any one of the previous three seasons. The ratio of neck to bottom infections as shown in some cases in 1915 was about as 13 to 8.

ON THE SEED HEADS.

The onion *Botrytis* has been found fruiting on the dead leaves, scapose stems, and the base of the seed stalks in a number of onion seed fields visited. In several instances the growers complained that the seed heads had been blasted. A careful examination of these heads showed that invariably the seed stalk, or seed head, or both, had been attacked by the neck-rot fungus. The heads showed the coarse, branching mycelium of the fungus upon and in the dead floral parts. Isolations showed it to be the onion *Botrytis*. A species of *Cladosporium* was found quite frequently upon the dead seed heads but inoculation experiments, reported elsewhere in this paper, showed the *Botrytis* to be the cause of the blighting or blasting of the seed heads attacked. At the base of the seed stalks where the fungus attacked the leaves the disease appeared to be more prominent where the leaf sheath clasped the stem and on old remnants of leaves adhering to the stem or seed stalk. Fig. 1, plate VIII, shows two diseased and one healthy, normal seed heads. The head partly open shows the fungus fruiting just beneath the split in the membrane enclosing the flowers. The diseased seed head which is open shows the fungus fruiting upon the flower pedicels. Fig. 2, plate VIII, shows a seed head which has been attacked by the neck-rot fungus (blasted). The black fungus bodies on the stalk are due to the presence of a species of *Pleospora* which appears after the seed stalks are dead.

ETIOLOGY.

INFECTION OF DRY BULBS BY DUSTING WITH DRY SPORES

During the work of going over hundreds of crates of red and yellow onions stored in various storage houses and in which the *Botrytis* fungus was constantly associated with the disease, in but very few instances was evidence obtained that the fungus passed from onion to onion except where the bulbs had been wounded either during harvesting or storing, or by being bruised while screening. The dry skins or scales surrounding the healthy bulbs appear

to be a barrier to infection. However, it must be conceded that infection of healthy bulbs may take place where they are covered by a mass of wet, sprouting and rotting specimens. In order to test experimentally the possibility of infection thru dry skins, large sound onions were cleaned aseptically, by carefully removing the outer dry scales with sterile forceps, then placed on crystallizing dishes in deep preservation jars kept moist by the use of wet cotton in the bottom of the jars. Some of the bulbs had one of the outer dry skins intact while on others these were removed, exposing the fresh fleshy scales. These onions were divided into two lots; on some of each lot spores in sterile distilled water were placed upon the fleshy and the dry scales, while on others the dry spores were dusted upon the scales. Suitable numbers of each lot were left untreated as checks. Upon examination later it was found that none of the dry spores placed upon the dry, firm, fleshy scales had germinated; but in the cases in which the spores were suspended in drops of water, germination had taken place on both the dry skins and on the fleshy scales. Infection of the tissue followed on only a few of the fleshy scales. The extent of infection of the fleshy scales was especially marked where a small wound was present. The mycelium penetrated thru the fleshy scales discoloring the tissue and killing the cells. Harter (1916) reports that in his study of the storage rots of economic aroids it was found necessary to apply sterile water once or twice to the tubers and corms after inoculation with the fungi studied, but that no further applications of water were necessary after decay had once started. It was plainly evident from these experiments that drops of water, or sufficient moisture, must be present to bring about germination of the spores, and later, infection of the bulbs.

INFECTION OF GROWING PLANTS BY MEANS OF DRY SPORES.

All attempts to bring about infection of normal green and uninjured leaves and necks of onion plants by dusting dry spores over them were unsuccessful except when small drops of water were supplied, and the plants covered with bell jars for 36 hours. On very sunny days when only a small amount of moisture was supplied infection did not follow. In other words, infection from spores was successful only when the surrounding conditions were very

similar to those which obtain in the onion fields when the plants are wet with dew or rain for two or three days and cloudy weather prevails.

INFECTION OF GROWING PLANTS BY MEANS OF SPORE SUSPENSIONS.

On July 8, 1915, twenty-three onion plants, started from white onion sets and growing in sterilized soil in three-inch pots, were placed in a large inoculation chamber. These plants were then thoroly atomized with a suspension of the onion *Botrytis* spores in sterile water. After inoculating the plants in the chamber, three additional plants which had not been atomized with spores were added to the lot to serve as checks. On July 12 every inoculated plant showed infection from the *Botrytis* on some of its parts. A number of the leaf tips were dead and showed abundant infection when examined a few days later. Every inoculated plant showed the fungus fruiting on the neck, in most cases at the edge of the leaf sheath at a point where it clasps the neck. The tissue at that point appeared soft and water soaked and of a faint yellowish color. Possibly the outer leaves may have been slightly on the decline, that is, beginning to yellow at the time of inoculation, but this certainly was not apparent. On these plants the fungus clearly showed varying degrees of parasitism, being able to attack the green tissue and also to live on the softened tissue. Certainly, the host tissue was killed in advance of the hyphae. From the behavior of the plants it was plainly evident that most of the infections occurred thru the neck of the bulb and not thru the upper portions of the leaves. The uninoculated checks remained healthy. After these plants were killed by the onion *Botrytis* and had fallen over to the ground the loose-growing, common *Botrytis* appeared on some of them as a saprophyte on the dead plants. This experiment was repeated a number of times with similar and convincing results, which proved conclusively that it is comparatively easy to bring about infection of growing onion plants. Apparently the only limiting factor is that the drop of water or suspension does not dry off too quickly.

On April 29, 1915, a deep, greenhouse flat containing 15 onion plants growing vigorously in sterilized soil was placed in the inoculation chamber with a fairly high relative humidity, and atomized with

a spore suspension of the onion *Botrytis* in prune juice. The spore suspension was made by pouring a tube of sterile prune juice upon an agar slant culture which was fruiting profusely and after rubbing off the spores and possibly some pieces of the aerial growth with a sterile platinum needle the liquid was poured into the receptacle of a De Vilbis atomizer. When atomizing the suspension upon the plants some small pieces of the mycelium came up thru the atomizer. Later all of these produced infection wherever they struck. Three days after atomizing, a considerable number of the leaves showed small white spots about 1 to 2 mm. in length (for they had a tendency to run lengthwise of the leaf) scattered over the leaf. By carefully peeling off the outer epidermis of a leaf a typical *Botrytis* mycelium, 6.6 microns wide, was found penetrating the tissue and growing along the leaf in the direction of its growth. Other typical infections revealed a similar condition. The chlorophyll quickly disappeared from these spots. After remaining in the moist atmosphere of the greenhouse for six days the plants went down rapidly, the fungus fruiting abundantly on the dead and dying, water-soaked-appearing plant parts. After a lapse of one month all of the plants placed in the box were dead, the tops remaining as dried material covered with abundant conidia. It was also evident from this experiment in which sterile prune juice was used instead of sterile water as a medium in which to convey the spores to the plants, that infection takes place readily from the drops of spore suspension.

Ward (1888) in his infection experiments with the conidia of the *Botrytis* causing a lily disease noted that the young germ tubes, altho unable to infect green leaves and bulbs, made a stronger attack if some saprophytic nourishment was at hand. In order to determine whether there was any difference in the rate of infection from conidia when placed in either sterile water or prune juice and then sprayed on the plants some experiments were made. One of these was as follows: Thirty healthy onion plants about 6 to 8 inches high and growing in a greenhouse bench at the Geneva Station were covered with large glass lantern globes. Ten of these plants were then atomized with the onion *Botrytis* spores suspended in water, ten with spores in prune juice and ten were retained as checks. All of the glass globes were then closed at the top by wrapping cotton loosely about the projecting leaves and covering the remaining space with a glass

plate. Five days later there were unmistakable signs of infection on both the prune-juice-sprayed and the water-sprayed plants, but it could not be seen that the rate of infection was any greater in the case of the prune-juice-sprayed plants except possibly in one or two instances. Typically some of the infections took place on the leaves about the necks and thru those which were very slightly yellowing. At this point it is well to call attention to the work of Brooks (1907, 1908) who found that the conidia of *Botrytis cinerea* were able to infect lettuce leaves which were slightly yellowing, that is, in the incipient stages of decay, while they were unable to attack normal green leaves. This author suggests three explanations for this phenomenon.

In another series of tests comparing prune juice and water as a medium in which to convey the spores to the plants, prune juice gave a slightly greater percentage of infections. Even tho infection of growing plants took place readily when drops of water were present it was evident that the results of these tests were slightly in favor of the prune juice suspension. Apparently, a slight amount of nutrients in the spore suspension has an influence upon the rate of infection.

EFFECT OF SIZE OF DROP OF SUSPENSION UPON INFECTION.

In an attempt to determine the effect of size of drop of spore suspension upon infection of the onion eight healthy plants were selected. Three of these were atomized with spores of the onion *Botrytis* in water, the atomizer covering the plants with a very fine mist. Three of the plants were sprayed with an onion-*Botrytis*-spore suspension using a tin atomizer of the hinge type which placed the suspension on the glaucous leaves of the plants in small drops very similar to drops of rain or dew. Two plants were retained as checks. All of the plants were then covered with bell jars. In the course of six days the inoculated plants showed abundant infection on the leaves and stems. The checks remained healthy. While there was not a marked difference shown in amount of infection thru either the small or the large drops, it was clearly demonstrated that the drops of liquid must be of sufficient size to prevent their drying off quickly, a condition such as obtains in the onion fields during a wet season. Observation has shown that following such a season there is considerable neck rot of the bulbs in storage.

INFECTION BY PLACING SPORES BETWEEN LEAF SHEATH AND
NECK.

The possibility of infection taking place by dry spores dropping down in the leaf sheath was, in one instance, tested as follows: Eighteen apparently normal onion plants growing in a greenhouse bench were selected; then, with a sterile platinum needle, dry spores from a pure culture of the onion *Botrytis* were carefully placed down in the leaf sheath, where it clasps the neck, the leaf being gently drawn aside during the operation. Some of the leaves fitted tightly while others did not. Untreated plants were retained as checks. Fifteen days from the date of inoculation seven of the fifteen plants showed infection, that is, the leaf was wilted, drooping, and appeared as if it had been scalded. On eight of the plants no noticeable infection had taken place. Thirty days from the date of inoculation nine of the fifteen plants showed severe injury from the *Botrytis* infection. The leaves were dead and nearly dried up and the fungus mycelium was found to have passed down into the necks of the bulbs. The checks remained healthy. It was evident from these and similar tests that the dry spores lodging in the axils of leaves or in the leaf sheath very readily found moisture conditions suitable for germination and later caused infection. It seems fair to assume that this may happen when spores of the neck-rot fungus from piles of decaying onion refuse near onion fields are air borne and find lodgment on the plants of the growing crop.

INFECTION THRU INCISIONS.

Some preliminary experiments designed to test the possibility of securing infection by placing the *Botrytis* spores in incisions in the side of the bulbs and necks showed conclusively that infection takes place very readily thru such wounds when they are covered with wax or some other similar material to prevent a drying out of the exposed tissue. Following the preliminary experiments eighteen onion plants which had formed bulbs about two inches in diameter were selected. After cleaning the neck with a cloth soaked in mercuric chlorid solution and then washing off with sterile water, an incision was made into the neck about one and one-half inches above the surface of the soil. Dry spores from a pure culture of the onion fungus were then placed in the incisions which were immediately covered with grafting wax. Spores were omitted

from some of the incisions, these to serve as controls. Seven days after inoculation every inoculated plant, with a single exception, showed that the fungus had made an attack upon the tissue of the neck. All of the control plants remained healthy. The infections showed as a whitening of the tissue around the point of inoculation. In some cases the diseased area extended one-half to three-fourths inch above the wax covering, and on some leaves the whitened or scalded appearance extended up along the leaf, in one case to a distance of three inches above the point of inoculation. In the course of twenty-one days the disease had progressed to the point where nearly every inoculated plant was completely killed. An examination of the leaf tissue about two inches above the point of inoculation showed it to be completely filled with the fungus mycelium, making it impossible to determine whether it was inter- or intracellular. The tissue became soft and of a pale yellowish color above the areas in which the mycelium could be found. Evidently infection takes place readily thru wounds of this type.

INFECTION BY MEANS OF SCLEROTIA.

That infection of bulbs can take place by means of sclerotia was clearly shown by making incisions into the sterilized sides and necks of healthy bulbs and inserting into the wounds sclerotia picked from pure cultures. These were then covered with wax. The results with bulbs so inoculated were positive while the checks which received no sclerotia gave negative results: Evidently, sclerotia also must be looked upon as a source of infectious material.

INFECTION THRU THE FLOWERS.

While working with inoculation experiments upon the leaves, necks and bulbs of the onion in the Geneva greenhouse a number of seed bulbs or "mother bulbs" placed in a bench produced seed heads. It was noticed that as soon as the flower heads opened, some of them were attacked by the onion *Botrytis* which was fruiting upon onion stems in the same house. The young germ tubes readily attacked the stigmas, anthers and petioles of the flowers producing a blasting of the flower heads very similar to that which occurred in the onion seed fields.

In experiments designed to test the pathogenicity of the onion *Botrytis* for the flowers, dry spores placed in freshly opened flowers

germinated, and readily attacked and destroyed the non-cuticularized floral parts. The fungus then worked down thru the receptacle and invaded the seed stalk. In the greenhouse, at least, the initial infection of the flowers took place by means of wind-borne spores from diseased plants in adjoining benches. It seems fair to assume that infection of flowers in the onion seed fields could occur in a similar manner. Field observations and all indications confirm this idea.

MYCELIUM IN NECKS OF INFECTED BULBS.

At the conclusion of the greenhouse experiments in 1915 there was a considerable quantity of onion bulbs at hand some of which had matured. Some of these bulbs had shown infection from the neck-rot fungus early in the season, but after the neck and some of the dry, outer skins had been removed the specimens appeared sound even tho somewhat undersized. A careful examination was made of a considerable number of these bulbs in search of the mycelium in the necks. In nearly every instance the mycelium was found between the outer three or four fleshy scales at the necks of the bulbs. In the case of one apparently healthy bulb a mat of mycelium was found between the second and third fleshy scales where it had caused no discoloration of the tissue. Isolations from this mycelium gave a prompt and vigorous growth. When such bulbs were placed in a moist chamber the neck rot developed rapidly.

INOCULATION EXPERIMENTS WITH OTHER PLANTS.

Species of *Lilium*, including *L. tigrinum*, *L. candidum*, and *L. grandiflorum* were dusted with dry spores and sprayed with various spore suspensions of the onion *Botrytis* on leaves, buds, and open flowers. These gave negative results in every instance, indicating that under the varying conditions under which the tests were conducted the onion *Botrytis* is not pathogenic for these species of *Lilium*.

Greenhouse flats of lettuce plants were placed in an inoculation chamber, at a number of different times, and sprayed with spores of the onion *Botrytis* suspended in sterile distilled water. In none of the trials did infection from the onion *Botrytis* follow the inoculations.

Garden peas were started in greenhouse flats filled with sterile sand from a cutting bench. These peas were started for the purpose of testing germination and were planted very close together. When the plants were about five inches high the flats were placed in a moist chamber and atomized with a spore suspension of the onion *Botrytis*. No indications of infection were apparent until six days later when it was observed that infection had taken place only on the yellowing leaves and stems. After infection had taken place the infected parts became flaccid and water soaked. The fungus then progressed to other parts of the plants killing the tissue in advance of its growth. Attention has already been called to the observations of Brooks (1908) who noted a similar instance in the case of *Botrytis cinerea* on lettuce plants.

SUMMARY AND CONCLUSIONS.

The results of inoculations of dry onion bulbs, and of growing plants, by dusting with dry spores, were negative. However, positive results were obtained from inoculations made in the following ways: (1) By spraying spores on the growing plants, and on the bulbs; (2) by placing dry spores between leaf sheaths and the neck; (3) by placing spores in incisions in the necks and sides of the bulbs; (4) by placing sclerotia in incisions in the necks and sides of bulbs; and (5) by dusting dry spores on the freshly opened flowers. Evidence was secured that drops of liquid in which the spores were suspended must be of sufficient size to prevent their drying off quickly. Some evidence was also brought forth which indicated that a small amount of nutrients in the drop of spore suspension slightly favors infection. An examination of bulbs from plants used in the infection experiments showed the mycelium of the fungus to be present in the necks.

A summary of the inoculation experiments brings forth but one conclusion, viz.: That spores in large drops of water (or sufficient moisture) on leaves, in wounds, and chiefly on the necks of onions (and only onions) produce the typical neck-rot disease. Sclerotia must be looked upon as a source of infectious material. The leaves attached to and clasping the lower part of the neck are very susceptible to attack, especially when they are on the decline, i. e., slightly yellowing.

With the results of the inoculation experiments in mind it was possible to go into the onion fields and storage houses and explain most of the conditions found there. It is believed that in the field the greater part of the infections above ground take place thru the lower leaves and the neck and then work down into the bulbs, the mycelium going into the storage houses in the necks of the bulbs, where it develops rapidly under favorable conditions.

THE PARASITE.

MORPHOLOGY.

Mycelium.—The mycelium is large and coarse, septate, and varies considerably in diameter in the same hypha. In diseased onion bulbs the mycelium frequently occurs as only 4.5 microns wide, while in diseased seed stalks from the field it is often 9 microns wide, the average width being approximately 6.5 microns. It has no regular method of branching, and is not only intercellular but intracellular, it being impossible in many cases to determine this point because of the disintegrated condition of the host tissue (Plate II, fig. 11). In the bulbs and necks of the host plant the young mycelium is practically colorless. With age the walls become of a sepia tint bordering on a light brown color. Various types of cross connections may be found in the host tissue (Plate II, fig. 12). The mycelium when crowded down between the bulb scales forms a dirty-white mat. Here, in one instance, it gave an average width of 6.3 microns for a large number of measurements. The mycelium is filled with a dense, fine-grained or minutely vacuolate protoplasm, frequently appearing granular. When grown in solid media weak in nutrients, and in synthetic solutions, the mycelium shows considerable variation in form and method of growth. Fig. 14 on Plate III, shows a type of mycelium common in solid media. A type of heavy mycelium is frequently found in pure cultures (Plate III, fig. 26). Sporulating filaments not unlike those shown by Beauverie and Guilliermond (1903) and giving the same staining reactions to haematoxylin and methylene blue were found (Plate IV, figs. 33, 34). The mycelium showed "corpusecular bodies" similar to those shown by these investigators (Plate IV).

The short, erect conidiophores arise directly from the mycelium either singly or in clusters (Plate II, fig. 6). They turn from hyalin to light brown and later to brownish black. Upon the host plant

the conidiophores are usually unbranched (Plate II, figs. 6, 9), while in cultures there appears to be a tendency toward branching (Plate II, fig. 8). The compact clusters of conidia are borne upon vesicles which have a tendency toward a spiral arrangement (Plate II, fig. 7), and usually upon the upper portion of the conidiophore (Plate II, fig. 10).

Appressoria.—While studying the behavior of the fungus as it worked its way down the neck of inoculated onion plants it was noted that appressoria or organs of attachment were formed rather profusely. These appeared to have an important relation to the discoloration and solution of the cell walls of the host. The writer's attention was first attracted to these bodies while examining an onion leaf which had been sprayed with a spore suspension of the onion *Botrytis*. The leaf appeared green and perfectly normal with the exception of small white spots where infection had taken place, and one particular area where the fungus was fruiting abundantly. Around this latter spot the leaf tissue for a distance of about 5 mm. appeared as if it had been scalded. By making thin sections thru the leaf, at a point where the diseased and healthy areas adjoined, and then unrolling the sections, the inner side of the leaf tissue could be examined. This examination revealed a profusely branched mycelium growing upon the inner epidermis of the leaf and extending along in the same direction as the long diameter of the cells, i. e., in the direction of growth. A considerable number of the tips of the hyphae showed knobs or enlargements of various shapes and sizes, many of them being simply enlargements of the tips of the hyphae (Plate III, figs. 15-22). In most cases these bodies were full of dense, bright protoplasm, while the mycelium just back of them was vacuolate, and markedly so. The tips of these bodies were surrounded by a film of material which was very refractive to light and which could be rather easily demonstrated by staining in weak gentian violet for a few seconds. This condition as described was found to be very common in the inoculated plants and those showing natural infection from the field. Quite frequently the mycelium thickens slightly just back of the appressorium. The young mycelial elements may also become markedly flattened where they come in contact with the culture dish (Plate III, fig. 32). The bodies appear simply to apply themselves to the host tissue following which there is a softening of the tissue and it takes on a water-soaked appearance.

The chlorophyl disappears and the mycelium branches and penetrates the tissue at will. It was found, however, that similar, but more complex and larger organs of attachment were formed when the fungus was grown upon culture media. Here they may be formed as single flattened hyphal tips where they come in contact with the glass side of the culture dish (Plate III, fig. 30), or as a repeatedly branched tassel-like cluster of hyphae with their tips flattened against the glass walls (Plate III, figs. 25, 27, 31). In many instances two or three hold-fasts could be seen close together, apparently due to the branching of the mycelium just above (Plate III, figs. 28, 29). In the center of many of the hyphal tips as they come in contact with the glass a bright, clear spot is to be seen, while around the thickened hyphal walls a brownish film of a mucilaginous nature is visible. Ward (1888) and Kean (1890) studying a lily disease caused by a *Botrytis* observed a "glairy film" around the tips of the short hyphae forming the organs of attachment and concluded that this was a ferment excreted by the tips of the hyphae. They point out that these are the bodies described by De Bary (1884) and called by him "Haft-organen."

Aderhold (1896) mentions the fact that the appressoria of *Venturia* are frequently surrounded by a zone of mucilaginous material. Voges (1910) also noted in *Venturia* a "gelatinous envelope" around the appressoria of that fungus. He suggests that these are functional in attachment of the fungus. Smith (1900), Price (1911) and Cook and Schwarze (1913) figure organs of attachment of *Botrytis* spp., not greatly unlike those of the onion *Botrytis*.

Blackman and Welsford (1916) have demonstrated that the germ tubes of *Botrytis cinerea*, when they have reached sufficient length (about one spore-diameter), "possess a mucilaginous investment" by means of which the germ tube is aided in exerting sufficient pressure to penetrate the leaf tissue. These authors further state that this method of penetration is impossible in the absence of the mucilaginous investment of the germ tube, but that "penetration can occur without the development of an appressorium."

Sclerotia.—The typical, firm, black sclerotia may be found upon any or all parts of the onion bulbs in storage. These vary in size from 1 mm. in width to large crusts covering nearly the entire side of a bulb (Plate VII). The sclerotia vary in shape from small roundish bodies 1 to 2 mm. wide and flattened on one side to large,

contoured and folded crusts covering the diseased bulbs. Frequently there are indications of striations on the sclerotia. These run in the same direction as the veins of the bulb scales. It is not uncommon to find the sclerotia massed together in the form of a sclerotial crust encircling the neck of the bulb. Sclerotia were found which presented the appearance of an inseparable layer of dull black enamel over the onion scales. Typically, the sclerotia have a tendency to round off on the upper surface while the lower side is flattened or even slightly hollowed out and inseparable from the onion bulb scales or tissue. When found between the bulb scales they often take the shape of the cavity which they fill. The sclerotia are of the cartilaginous type, having a hard outer cortical covering or layer filled with a white mass of mycelium closely pressed together forming a pseudoparenchyma. Cultural experiments, to be discussed later, show that the presence of a certain amount of moisture is favorable to the development of sclerotia. Sclerotia are not formed readily in cultures except where certain conditions prevail. They are formed where the mycelium is pressed together at the point of contact of fungus and glass, very often appearing as centrifugally formed crusts. It was observed that in many cases the appressoria are incipient stages of the sclerotia (Plate III, figs. 23, 24). At first the sclerotia are dark flesh color rapidly becoming black.

The sclerotia germinate by the ends of hyphae pushing out from the pseudoparenchyma and forming tufts of conidiophores bearing conidia similar in shape, size, color, and germination to those found on the mycelium (Plate II, fig. 13). All attempts to induce the sclerotia to germinate in any other manner than by pushing out typical conidiophores which bear conidia or by the formation of a mycelium were failures with the exception of one remarkable instance which will be described in a later paragraph. No apothecia were formed on any of the sclerotia. Sclerotia taken from onions in various storage houses and from pure cultures were sterilized in several different ways, then placed in test tubes of sterile soil, in deep and shallow dishes of sterile sand and loamy soil, and kept in the laboratory, out-of-doors, and in the ice box for varying lengths of time; but in all cases the tufts of conidiophores were produced when germination took place. Sterilized sclerotia which were thrust i to sterilized cornmeal moistened with water, produced, in germi-

nation, an abundant mycelium which gave a fairly profuse crop of sclerotia thruout the medium. In other experiments sterilized sclerotia were floated upon sterile water and prune juice. Some of the sclerotia sank to the bottom of the liquid; but many of those which remained at the surface produced conidiophores and conidia. When the sterilized sclerotia were thrust into prune, potato, and onion agar in petri dishes, pure cultures of the fungus were invariably obtained.

A number of sclerotia were separated into two lots before sterilizing — one lot comprising the small sclerotia, the other the larger ones. When placed on moist sterile sand to germinate both sizes responded with the usual crop of conidiophores and conidia. However, it was noticed that the small sclerotia were slightly more viable than the larger specimens.

Sclerotia subjected to the stimulating action of drying, and to fumes of chemicals such as ether, chloroform and alcohol gave results no different from those previously noted.

From the germination tests made upon hundreds of sclerotia the following points were secured: The most rapid and the highest percentage of germination occurred when the sclerotia were kept out-of-doors or in an ice box. A fairly low temperature (about 10° C.) greatly stimulates germination. Drying and exposure to sunlight reduced the viability of the sclerotia. If the sclerotia placed on sterile, moist sand or similar media did not germinate, or even after they had produced a crop of conidiophores, they readily broke down leaving the outer cortical layer in the form of a crust.

The length of time during which the sclerotia remain alive is very important. A fairly large percentage of the sclerotia were found to be dead at the end of one year. One lot of sclerotia kept for two years in the laboratory showed a very weak germination in a very few instances at the end of that time. Results of the tests with sclerotia indicate that under average conditions the life of the sclerotium in moist soil is but a few months, possibly only a few weeks.

It was noted above that in one particuar instance germination took place other than by the production of the typical tufts of conidiophores or the formation of mycelium. This was the case with a lot of sclerotia picked from onion bulbs in storage early in 1913. These were sterilized by being soaked in sterile distilled

water, then immersed in a 0.1 per ct. mercuric chlorid solution for two minutes, and again washed in sterile distilled water. The sclerotia were then picked from the water with sterile forceps and placed upon moist sterile sand in petri dishes. Some of the dishes were kept in the laboratory in a dark closet, the others were placed in an ice box. On June 6, 1913, it was noted that two of the plates in the ice box showed that germination had taken place by the production of tufts of conidiophores bearing conidia, while upon a third plate there were no typical conidiophores but instead there were formed stalk-like bodies with masses of conidia at their tips. (Fig. 1, plate X). The stalks of these peculiar structures were black while the tips were white. These bodies were 20 mm. high and 3 mm. wide. Cultures made from the knobs of these structures upon onion agar gave pure cultures of the *Botrytis*. The conidia found on these knobs gave an average measurement of 11.3 microns long and 5.7 microns wide. A further analysis of these structures showed the stalk of one of them to be made up of 16 conidiophores. No further development was noticed in these structures.

Conidia.—The pale colored or hyalin conidia are borne upon stout, short, erect conidiophores, these being very rarely over 1 mm. high, usually much shorter. The conidia are oblong to elliptical in form, often slightly tapering at both ends (Plate I, fig. 1). The short tip or sterigma may often be seen at the narrower end of the conidium. The conidia have been found to vary in size from 7.1–16.2 x 3.8–6.3 microns, the average from hundreds being 10.3 microns long and 5.1 microns wide.

The conidia fail to germinate in a saturated atmosphere. In water they are wetted with difficulty, but soon absorb water and germinate rapidly, usually by one, occasionally by two, germ tubes, forming considerable mycelium in 24–36 hours (Plate I, fig. 3). The mycelial branches unite very frequently, forming unions (Plate I, fig. 4), also the tips of hyphae form cross connections (Plate I, fig. 5).

While examining some old cultures of the fungus there were found at the bottom of the slant in the test tube, round, dark brown conidia about 3 microns in diameter and having very thick walls, while at the upper part of the slant typical elliptical conidia were formed. It appeared that conditions of nutrition caused the formation of these peculiar conidia. Price (1911) described some peculiar spore

forms of *Botrytis*. Perhaps these round conidia observed by the writer are similar to the microconidial form of fructification mentioned by Istvanffi (1905), and Brooks (1908) who worked with *Botrytis cinerea*.

Comparative morphology.—The cultural characters of the onion *Botrytis* were critically compared with those of other forms or species of *Botrytis*, since during the course of the investigation of the onion *Botrytis* fungus the writer had opportunity to isolate a considerable number of forms or possibly species of *Botrytis* from a number of hosts. Some of these were living saprophytically on dead plant parts, while others were active parasites. At the outset, the possibility of the onion *Botrytis* being the common gray mold found upon greenhouse plants presented itself. As soon as the onion *Botrytis* was placed in pure culture and compared with the so-called *Botrytis cinerea* Pers., it was plainly evident that the organisms compared were entirely distinct species. The onion *Botrytis* possessed a low, dense, felt-like aerial mycelium bearing elliptic, smoke-gray conidia on very short conidiophores, while *B. cinerea* showed the loose, cottony, cinereous mycelium with long erect conidiophores, bearing the pale, globose conidia (Plate I, figs. 1, 2). The writer has isolated the loose-growing common *Botrytis* from dead onion leaves and from the tips of growing onion leaves where dying back had taken place due to the disease called "white tip." It was also isolated once from onion bulbs stored in a storage house along with a quantity of carrots which were severely attacked by *Sclerotinia libertiana* and overgrown with *Botrytis*. In another instance both the common *Botrytis* and the onion *Botrytis* were isolated from the perennial or tree onion growing in a private garden. Here, the onion *Botrytis* was acting as a destructive parasite killing the lower leaves and necks of the clusters of green bunching onions, while the common *Botrytis* was living as a saprophyte on the dead onion leaves. In Plate IX the onion *Botrytis* is shown as culture 12091 with its low form of growth, and the common *Botrytis* as culture 12091-1 with its loose, cottony mycelium completely filling the culture tube. Both cultures are of the same age and on the same kind of medium. Plate IX also shows four cultures of *Botrytis* picked at random from stock cultures. The cultures serve to show the dense, felt-like growth of the onion *Botrytis* as compared with the loose, cottony growth in the three other cultures, and also of

that of a large number of isolations of *Botrytis* from a number of greenhouse plants. The writer will not venture a statement as to the classification of species of *Botrytis* showing the loose, cottony growth. While greatly resembling each other, it may be said that they present some distinctive cultural characteristics. The onion *Botrytis* has been carefully compared with forms of *Botrytis* from every available source among which were *B. parasitica* said to be the cause of the "Dutch bulb disease," and *B. peoniae* the cause of the *Botrytis* disease of peonies. None of these seems at all similar to the onion *Botrytis*. To summarize, the writer is confident that the onion *Botrytis* is responsible for the neck-rot disease of onions and is a separate species. Smith (1900, p. 397) appears to be the first in America to point out that the *Botrytis* causing the onion disease "was not a typical cinerea" because of its "low, dense manner of growth." He gives some excellent illustrations of the conidia and conidiophores of the onion *Botrytis* comparing them with *Botrytis cinerea*.

The name of the fungus.—Considerable confusion has existed as to a name for this fungus since it has apparently been known under a number of different names. Sorauer (1876) appears to be the first to record a disease of onions which may be the neck-rot disease. In 1886 the same author described a disease of onions due to a *Botrytis* which evidently included a sclerotial stage. He referred the *Botrytis* stage to *Botrytis cana* (Pers.) Fr., and the sclerotial stage to *Sclerotium cepae*. Again, in 1908, Sorauer discussed a *Botrytis* disease of onions and referred the *Botrytis* stage to *Botrytis cana* Kze. et Schm., and the sclerotial stage to *Sclerotium cepivorum* Berk.

Frank (1880, 1896) reported a *Botrytis* disease of onions. He reiterated Sorauer's statements as to the possible identity of the *Botrytis* stage of the fungus with *Botrytis cana* Pers., and the sclerotial stage to *Sclerotium cepae* Berk. et Br. This author (1896) stated that he noted a *Botrytis* on *Allium ursinum* near Leipzig which was similar to that observed by Schröter.

Halsted (1890), in what appears to be the first report in America of an onion bulb disease due to a *Botrytis*, states that *Botrytis parasitica* Cav. may be the cause.

In 1894, Massee, writing in the *Gardeners' Chronicle*, described an onion disease due to a *Botrytis* with an ascogenous stage. This

disease, he states, was due to *Sclerotinia bulborum* Wakker. He figures a *Peziza* form not unlike *Sclerotinia libertiana* Fekl., and a *Botrytis* form with globose conidia very similar to *Botrytis cinerea* Pers. Oudemans (1884) and others state that no *Botrytis* stage has been found for *Sclerotinia bulborum* (Wakk.) Rehm., which was described by Wakker (1883) as causing a disease of hyacinth and other bulbs, and that none occurs. At any rate the description and figures presented by Massee cannot apply to the fungus causing the neck rot of onions. Again, in 1903 and 1914, Massee restated that a destructive rot of onions due to *Sclerotinia bulborum* occurs. Until the connection between the *Botrytis* and the *Sclerotium* forms has been demonstrated these descriptions only lead to endless confusion.

In 1900 Smith pointed out that the onion bulb rot due to a *Botrytis* and observed by him near Munich was not typical *B. cinerea* because of its low, dense manner of growth and smaller spores. This author also doubted the possibility of the onion rot being due to *Sclerotinia bulborum* Wakker as suggested by Massee.

Voglino (1903) made a careful study of a disease of *Allium sativum* which occurred in Italy and was able to prove conclusively that the onion disease caused by a *Botrytis* as described by Sorauer (1886) and Prillieux (1897) is not due to *Sclerotium cepivorum* Berk., but he suggested that it may be a *Botrytis* described by Schröter on *Allium ursinum* and one found by himself as occurring on onions and referred to *Sclerotium ambiguum* Duby. This author stated that due to the kindness of Professor P. A. Saccardo he was able to examine in the Exsiccati of Libert¹ typical examples of *Sclerotium cepivorum* Berk. (*S. cepae* Berk. et Br.) and to determine the identity of these with those found on *Allium sativum*. This author further stated that the sclerotial form of Libert and of the specimens which develop in various parts of Italy are constantly spherical in form as is true of the form found by Garavaglio in rice plants and described by Cattaneo² under the name *Sclerotium oryzae*. These correspond to the description by Berkeley as reported by Fries³ and by Saccardo⁴ for *Sclerotium cepivorum* Berk. (*S. cepae*

¹ Cryptog. exs. Ard. n. 238.

² Saccardo, Syll. Fung. 14:1153.

³ Summa Vegetabilia Scandinaviae, page 478.

⁴ Syll. Fung. 14: 1151.

Berk. et. Br.). "Minutum, sphaeroideum, gregarium, atrum, tegumento distincte parenchymatico fuligineo, intus cellulis varie ramosis intricatis albis faretum." Voglino carefully worked out the conidial form of this fungus which was very abundant on the bulbils and which he described as *Sphacelia allii* distinguishing it by the following characters: "Effusa bulbos, *Allii sativi* crustaceo-pulvinata, roseola, sporophoris e strato prolifero oriundis, ramulosis, hyalinis, dense fasciculatis septatis, 40-50 microns longis; conidiis sphaericis, hyalinis, quandoque catenulatis, 1-guttulatis 3-4.5 microns diam." This important work performed by Voglino and to which attention has been called by Delacroix (1909, p. 401) and others, places beyond all doubt the possibility of the neck-rot disease of onions being due to the fungus *Sclerotium cepivorum* Berk.

Clinton (1903-1904) in reporting a Botrytis as causing a serious onion bulb rot of white onions in Connecticut suggested that possibly it is the same as the Botrytis bulb rot of onions and hyacinths in Europe, where it is known in its perfect form as *Sclerotinia bulborum* altho he stated that no perfect or ascospore stage has been noted in Connecticut. He further emphasized the possibility that it is not specifically different from the common Botrytis which is found so commonly on greenhouse plants. According to this author's description of the fungus and its behavior, supplemented by cultures which the writer has secured from diseased onions from Connecticut, no doubt is entertained but that it is identically the same fungus as is found in New York, Michigan, and elsewhere causing the neck rot of onion bulbs.

Bruck (1907) made note of a disease of onions in Europe due to a Botrytis with a sclerotial stage which he suggested is *Sclerotium cepae* Lib. This fungus was found both in the field and in the storage house.

According to Jarvis (1909) a disease of onions in the form of a rotting of the bulbs at the neck before and after storing occurred in Connecticut. This author referred the trouble to the same fungus (a Botrytis) as reported by Clinton (1903) and also recommended a similar method of treatment.

Selby (1910) discussed a "dry or black neck-rot" of onions which occurs in Ohio and causes heavy storage losses when white onions are stored. Altho he does not state that a Botrytis stage

occurs he figured a diseased specimen which is typical of the *Botrytis* neck-rot disease. He stated that the disease is due to *Sclerotium cepivorum* Berk.

In reporting for the state of Oregon, Jackson (1914) mentioned a serious storage rot of onions due to a *Botrytis*. The disease is known there as onion stem rot and gray mold, and is said to be favored by moist weather conditions, poorly ventilated storage houses, and immaturity of the stock.

According to Hanzawa (1914) a disease of onions due to a *Botrytis* which has a sclerotial stage occurs in Japan. Hanzawa figured a *Botrytis* with oval to oblong spores, and stated that they measure 8.4-16.8 microns long and 6.3-10.5 microns wide. The conidiophores are branched at the tips and are about 1 mm. long. He further stated that the fungus resembles *Botrytis cinerea*. The small, round or elliptical sclerotia germinate by forming the *Botrytis* stage but not the apothecial stage.

In a recent publication Humbert (1916) discussed the same onion disease as reported by Selby for Ohio at an earlier date. He stated that the disease is disseminated by means of spores, but does not intimate that the fungus is a *Botrytis*. However, he affirms that the causal organism is *Sclerotium cepivorum* Berk.

As far as the writer is able to determine from a thoro search of literature the following fungi from the genus *Sclerotium* have been reported upon species of *Allium*. These will be compared with the fungus studied:

1. *Sclerotium ambiguum* Duby.¹ For this species the sclerotia are described as being minute, ovate or orbicular, with the margins elevated, and having a dark interior. This species has no *Botrytis* stage and does not resemble the onion neck-rot fungus in the sclerotial stage.

2. — *S. Brassicæ* Pers.² which is reported as occurring on the scapes of *Allium victorialis* has no *Botrytis* stage, and has sclerotia which are oblong, flat, 4-8 mm. long, and the interior becoming black. Not at all similar to the onion *Botrytis*.

3. *S. cepivorum* Berk. (*S. cepae* Berk. et Br.)³ A discussion of this fungus has been given in a previous paragraph. Its minute,

¹ Duby, Bot. Gall. 2:875; Saccardo, Syll. Fung. 14:1150.

² Saccardo, Syll. Fung. 14:1164; Lindau, Rab. Krypt. Fl. 1^o:674.

³ Saccardo, Syll. Fung. 14:1151; Lindau, Rab. Krypt. Fl. 1^o:661.

round sclerotia which have as a conidial stage, a Sphacelia as found by Voglino (1903), exclude it from identity with the neck-rot fungus.

4.—*S. durum* Pers.¹ has sclerotia which are 4–6 mm. long, sometimes 12 mm. long and as wide, at first under the epidermis then becoming free. This species is reported by Lindau to occur on the stem of *Allium* sp.; also, that *Botrytis cinerea* is said to develop from the sclerotia. It is not similar to the onion *Botrytis* sclerotia since the latter are in most cases inseparable from the host tissue.

5.—*S. inconspicuum* Lib.² which is reported to occur on putrid onion bulbs has very small, brown sclerotia not at all like the sclerotia in question.

6.—*S. pulveraceum* Dur et Mont.³ has round-globose, small (0.2–0.5 mm.) sclerotia which have been found in the bulbs of *Allium pallens*. These cannot compare with the sclerotia found in the onion bulbs attacked by the neck-rot fungus because of their small size.

7.—*S. tulipae* Lib. var. *Hyacinthi* Guep.⁴ which is reported as occurring on the stems of *Allium vineale* has thin, ovate-oblong, minute sclerotia from which *Botrytis parasitica* Cav. is said to have been produced. Their occurrence on the stem, and their small size and thinness distinguish them from the sclerotia of the onion *Botrytis*.

Six species of *Botrytis* have been reported as occurring upon some parts of plants of *Allium* sp. These, also, will be critically compared with the onion *Botrytis* studied.

1.—*B. aclada* Fres.⁵ is reported as occurring on the dying scapes of *Allium*. However, it does not correspond to the onion *Botrytis* because it has ovoid-oblong conidia which are said to be one-half the size of those of *B. acinorum* Pers.; that is, 5–7 x 3.5–4.5 microns.

2.—*B. cana* Kze. et Schm.⁶ is reported to have large oval spores. According to Ward (1888, p. 360) *Botrytis (Polyactis) cana* Berk. (*B. cana* Schmidt) has conidia measuring 30–33 x 15–8 microns. The conidia of neither of these species compares at all with the long elliptical conidia of the onion *Botrytis*.

¹ Saccardo, Syll. Fung. 14: 1165; Lindau, Rab. Krypt. Fl. 1^o: 674.

² Saccardo, Syll. Fung. 14: 1151; Lindau, Rab. Krypt. Fl. 1^o: 662.

³ Saccardo, Syll. Fung. 14: 1151.

⁴ Saccardo, Syll. Fung. 14: 1172; Lindau, Rab. Krypt. Fl. 1^o: 677.

⁵ Saccardo, Syll. Fung. 4: 131; Lindau, Rab. Krypt. Fl. 1^o: 285.

⁶ *Myk. Hefte* 1: 83; Saccardo, Syll. Fung. 4: 129.

3.—*B. cinerea* Pers.¹ has been reported on *Allium* sp. The writer has found this species growing upon dead parts of onion plants in a number of instances; but its roundish, oval conidia are not to be confused with the long elliptical conidia of the onion *Botrytis* responsible for the onion neck-rot disease.

4.—*B. fulva* Link² which is reported as occurring upon the dead stems of *Allium cepa* has spherical, yellow-brown conidia measuring 4–4.5 microns in diameter and having fine spines.

5.—*B. parasitica* Cav.³ which is reported by Höhnelt as occurring on *Allium ursinum* has conidia which are ovate and large, measuring 16–20 x 10–13 microns, or nearly twice the dimensions of the onion *Botrytis* conidia.

6.—*B. vulgaris* Fr. var. *interrupta* Fr.⁴ has been reported as occurring on scapes of *Allium*. This species has globular-elliptical conidia slightly longer than those of the onion *Botrytis*. However, it has typical constricted or interrupted conidia, which definitely excludes it from being the onion neck-rot fungus.

From the foregoing statements it will be seen that the fungus causing the neck-rot disease of the onion has been incorrectly assigned under a number of different names none of which is available for it. Accordingly, the name *Botrytis allii* n. sp., is proposed with the following description:

Colonies dirty white shortly becoming smoke gray (Ridgway, 1912, pl. 46), old cultures approaching the drab of Ridgway, close, dense, feltlike; mycelium septate, coarse, with no definite method of branching, 4–9 microns in diameter, filled with fine-grained or minutely vacuolate protoplasm, giving rise to short erect conidiophores; conidiophores numerous, septate, erect, usually about 0.5 mm., rarely over 1 mm. high, occurring either singly or in clusters, not often branched on the host plant, but with a tendency toward branching in cultures, branches short and indicating a spiral arrangement, conidia-bearing on upper portion ($\frac{1}{2}$ – $\frac{1}{3}$), conidial clusters approximate, not widely separated; conidia hyaline or very dilutely colored, elliptical, often slightly tapering at both ends, 7.1–16.2 x 3.8–6.3 microns, average 10.3 x 5.1 microns, attached by a short

¹ Saccardo, Syll. Fung. 4: 129; Lindau, Rab. Krypt. Fl. 1⁸: 284.

² Saccardo, Syll. Fung. 4: 123; Lindau, Rab. Krypt. Fl. 1⁸: 280.

³ Saccardo, Syll. Fung. 10: 536; Lindau, Rab. Krypt. Fl. 1⁸: 292.

⁴ Fr. Syst. Mycol. 3: 398; Saccardo, Syll. Fung. 4: 129.

sterigma to the vesicles in small clusters; sclerotia dull-black, firm, white inside with a dark outer cortical layer which is usually very finely wrinkled or striated, flattened or concave on the side by which they are inseparably attached to the host tissue, strongly convex above, single sclerotia 1-5 mm. in diameter usually several to many united in clusters or large sclerotial crusts which may be several centimeters in diameter. Sclerotia germinate by hyphae which immediately produce conidiophores. No apothecia produced.

Hab. Parasitic on leaves, flowers, and bulbs of *Allium cepa*.

PHYSIOLOGY.

Sources of pure cultures.—The pure cultures used in these studies were obtained from a number of different sources, including isolations from onion bulbs from Bermuda, Connecticut, Massachusetts and Texas. Isolations were made from Michigan material in the form of flower heads, bulbs in the field and storage, overwintered necks in field, and perennial onions. A large number were made in New York from sclerotia, from bulbs in field and storage, from overwintered onion refuse in the field, from flowers and stalks from the seed crop, and from the necks of plants in the growing crop. Whenever possible the diseased plant part was placed in a damp chamber where a profuse production of conidia would take place. The conidia were easily picked off by touching the tufts of conidiophores with a drop of sterile water on a platinum needle and then transferring to onion agar. When diseased onion bulbs were used they were either split open with a sterile knife, or the outer scales peeled off and placed under a bell-jar in a fairly damp atmosphere. Here, abundant fruiting took place on the newly exposed surface and isolations were made as noted above.

Growth on vegetable tissue.—Of the various raw plant parts used for media, including sour and sweet apple, carrot, tomato, potato, pea stems, string beans and sterile onion bulbs, the last were by far the most satisfactory, giving the most abundant growth. The raw onion scales were prepared by carefully peeling off the dry outer scales, then immersing the bulb in a 0.1 per ct. solution of mercuric chlorid for two minutes to disinfect the surface. The outermost scale was cut off with a flamed knife. The remaining scales were then sliced off and dropped into sterile 100 cc. Erlenmeyer flasks. Very often it was difficult to get the scales free from micro-organisms. After

standing three days to test sterility the flasks were inoculated by dropping spores in upon the moist scales. Such cultures gave a prompt growth which was in full fruit in eight days, the scales being completely hidden under a dense, close-growing mycelium and multitudes of smoke-gray conidia. Where the flasks were loosely plugged and the media dried out readily, no sclerotia were formed, but a heavy crop of conidia was produced. All of the other raw plant tissues were prepared in essentially the same manner.

Conidia, and even bits of mycelium, when placed on slices of sterile raw carrot in a sterile moist chamber gave a very slow, scanty growth on moist pieces, but no growth where the tissues were not moist or had dried out.

It was noted that the growth taking place on cooked vegetables was usually many times greater than that upon raw vegetables. This was true of all the various kinds tested with the possible exception of the onion bulbs. On string beans immersed in mercuric chlorid, then washed in sterile water and clipped into short pieces, placed in small flasks and inoculated with spores, the first growth appeared on the cut surfaces then slowly developed over the uncut surfaces. On this raw vegetable the growth was very scanty and produced no conidia. On string beans placed in test tubes and autoclaved at $110^{\circ}\text{C}.$ for twenty minutes the growth was very profuse. A heavy crop of conidia was produced but no sclerotia were formed.

Cultures on pea stems, sterilized and raw, gave a very striking contrast. On the raw stems the *Botrytis* developed very scantily, while on stems sterilized in the autoclave for twenty minutes an abundant crop of conidia was produced. The color of the growth in these flasks was drab (Ridgway). Numerous organs of attachment were formed, but no typical sclerotia.

On sterile raw lettuce leaves in deep culture dishes no mycelial development took place except where abundant moisture was present in the form of drops. On lettuce leaves autoclaved in 250 cc. flasks a dense growth occurred. The conidia were typically smoke gray and very abundant. In the autoclaved lettuce flasks it was seen that the liquid below the surface growth turned black while the liquid in the uninoculated checks remained clear. This suggested the possibility of a biochemical change taking place in the inoculated flasks following the development of the fungus.

Pieces of the raw flesh of the apple, when taken under aseptic conditions and placed in flasks and compared with similar pieces in flasks sterilized in the autoclave at 115° C. for fifteen minutes, gave a marked contrast. On the cooked apple the growth developed rapidly producing a few conidia and numerous appressoria at the edges of the cultures where the growth came in contact with the flask. On the raw apple the growth was very slow and but few conidia were formed. The fungus changed the color of the apple tissue from a light flesh color to a brown. The aerial mycelium was dirty white color in raw apple cultures.

Raw, sweet apple flesh, aseptically prepared, was compared with similarly prepared raw, sour apple as a medium in flask cultures. The mycelial growth at first was slightly more abundant on the sweet apple, following which a more abundant conidial production took place. On the sour apple flesh very few conidia were produced but a considerable number of sclerotia 1 to 3 mm. in diameter were formed at the bottom of the flask where the pieces of apple were in contact with the glass and also at the edges of the pieces of apple. This apparent difference may have been due to a variation in the moisture content of the two varieties of apples, the sweet apples being more pulpy. The relation of the reaction of the medium to the development of the fungus is discussed elsewhere in this paper.

On blocks of raw potato tuber taken aseptically, but very slight growth took place, while on blocks of potato tuber autoclaved for fifteen minutes an abundant development of dense, close-growing, felt-like mycelium occurred. In the autoclaved potato flasks hundreds of small typical sclerotia varying in size from 1 to over 2 mm. in diameter were produced over the upper surface of the medium. Along the sides of the flasks, at least, all the intermediate stages between the holdfasts and the fully developed sclerotia could be seen.

The sclerotia which are formed at the bottom of the cultures are usually irregular in shape, appearing as sclerotial crusts and often filling and taking on the shape of the space between the pieces of potato tuber. Frequently one finds a row of typical sclerotia along the edge of a potato block where it touches the side of the flask, indicating that the mycelial strands when compressed or forced tightly together readily form sclerotia.

A quantity of small green-onion bulbs was sterilized in mercuric chlorid, then washed in sterile distilled water and placed in 500 cc. flasks. When these bulbs were inoculated with dry spores of the onion *Botrytis* the young germ tubes attacked the tissue of the bulbs reducing them to a soft brown mass upon which a profuse crop of conidia and sclerotia developed.

Growth on plant broths, and on solid nutrient substances with and without broths.—Broths from various plant parts were used alone and added to various solid nutrient substances. This series included: lima-bean broth, potato broth, apple broth, prune juice, plum juice; cornmeal alone and saturated with prune juice, apple and onion broths; and sterile bread crumbs saturated with prune juice, apple, potato and onion broths. There was little noticeable difference in the rate and manner of growth of the fungus on any of these media. The sterile cornmeal plus prune juice gave the best development of sclerotia, while the cornmeal plus potato broth presented the most unfavorable conditions for sclerotium formation. The bread crumbs plus onion broth gave the best development of sclerotia together with the most profuse crop of conidia. Organs of attachment were also abundant on this medium. It is worthy of note that the color of the growth on onion broth was similar to the typical smoke gray which characterizes the growth on the host. On all of these media, wherever the mycelium came in contact with the side of the culture flask black masses of organs of attachment were formed. When sufficient moisture was present sclerotia were formed wherever crowding of the mycelium took place. Many of the sclerotia germinated almost immediately after formation by sending out tufts of conidiophores which later bore conidia.

Growth on synthetic solutions.—An attempt was made to grow the fungus on Knopp's and Ushinsky's standard synthetic solutions and also upon Coons' synthetic solution (Coons, 1916, p. 752). Upon these solutions only a slight amount of conidium formation resulted. The growths obtained are of interest, however, since they showed development of abnormal forms of conidia, some of which were extremely long and misshapen.

Growth on various agars.—In addition to plain, nutrient-glucose, and starch agar, other agars were made by adding agar flour to various plant extracts. Those worthy of mention are apple, potato, prune, lettuce-leaf (with and without peptone), and onion agar.

The growth obtained on the plain, nutrient-glucose and starch agars were very scanty. Only a few conidia and organs of attachment, and no sclerotia developed. Of the other kinds of agar tried, by far the most typical and abundant conidial development took place upon the onion agar with tomato agar coming next in order. The growth on potato and prune agar was rather slow, and with very scant conidium formation on the latter.

Since the onion agar¹ gave the most typical development of the fungus and because of its ease of preparation it was used almost exclusively for carrying stock cultures and for growing crops of conidia and sclerotia for inoculation experiments.

While the results with these media are by no means complete, they indicate that moisture is a factor which very largely determines the formation or non-formation of sclerotia and also that abundant sclerotial formation and conidial production may not occur on the same culture.

A critical study of over 1000 cultures of the fungus shows that it possesses constant and easily recognized cultural characteristics on several media. Upon onion agar, which is typical of the host plant, the most distinctive and reliable cultural characteristic of *Botrytis allii* is its low, dense, felt-like, close-growing, dirty white aerial mycelium, which gives rise to short conidiophores bearing the elliptical smoke-gray (Ridgway, 1912) conidia. With age, the surface growth may become light drab (Ridgway) and even approach the drab of Ridgway. This characteristic persists for generations.

Thermal relations.—Determinations for the thermal death point (T. D. P.) were made with spores of the fungus, using the capillary tube method as described by Novy (1899). Thin, straight capillary tubes of even caliber were prepared, by drawing out to approximately 24 inches pieces of glass tubing about five inches long; these when carefully drawn provided four tubes. The usual method for determining the T. D. P. of fungus spores is to use tubes of culture medium which are immediately poured into petri dishes as soon as time and temperature exposure are secured. The spores of *Botrytis*

¹ The onion agar was made up by slicing 200 grams of clean sound onion bulbs into somewhat less than 500 cc. of water, cooking for a few minutes and steaming in the autoclave for 30 minutes at 110° C. The onion juice was then strained thru cotton and filter paper and added to 15 grams of agar flour dissolved in 500 cc. of water. The volume was then made up to 1000 cc. with water, filtered, tubed, and autoclaved for 15 minutes at 115° C. This medium gives a very slight acid reaction, frequently being but slightly above the phenolphthalein neutral point.

become wet with difficulty, but it was found that by pouring 10 cc. of sterile distilled water on an agar slant culture of the fungus which was fruiting freely, the spores were washed off readily and could be drawn up into the capillary tubes by touching the surface of the liquid upon which the spores were held in the form of a film. After being filled and sealed the capillary tubes were placed upon a rack made of wire netting which was then supported in a water bath kept at correct temperature by the aid of a thermometer suspended in the liquid. At the end of the time allotted for the various temperatures, a tube was taken from the rack, wiped dry, one end scratched with a file then snapped off and flamed. By holding the flamed open end in the end of an agar slant tube of onion agar while the closed end was passed thru a gas flame the contents of the capillary tube were ejected upon the agar slant surface. Spores germinate in about 36 hours and growth can be detected macroscopically. The results are shown in Table I.

TABLE I.—THE EFFECT OF TIME AND TEMPERATURE UPON SPORES OF THE ONION BOTRYTIS.

Temper- ature.	TIME IN MINUTES.				
	1.	2.5.	5.	7.5.	10.
<i>Deg. C.</i>					
35.....	+	+	+	+	+
40.....	+	+	+	+	+
45.....	+	+	+	+	+
50.....	+	+	+	—	—
55.....	+	—	—	—	—
60.....	—	—	—	—	—
65.....	—	—	—	—	—
70.....	—	—	—	—	—

T. D. P. = 50°C. for 10 minutes.
 + = growth in tube.
 — = no growth.

In order to compare the test-tube-media method with the capillary-tube method as described and used in the trials reported above, onion gelatin in tubes of equal diameter and thickness of wall was used. After inoculation with the Botrytis spores the gelatin was carefully brought to the melting point, then placed in the water bath along with control tubes and one tube of gelatin with a thermometer immersed in it. After the temperature had reached the

desired point in the control tubes the tubes were exposed for ten minutes with but very slight variation of temperature when the plates were poured and cooled immediately. The results of these tests, which compare closely with those of the capillary-tube method, are as follows:

- At 37° C. for 10 minutes — Copious germination on the plates.
 “ 40° C. “ “ “ — Fair germination on plates.
 “ 45° C. “ “ “ — A few colonies.
 “ 50° C. “ “ “ — Three colonies.
 “ 52.5° C. for 10 minutes — One colony.
 “ 55° C. for 10 minutes — No growth.
 “ 60° C. “ “ “ — No growth.

It was found by similar tests with pure cultures which had not sporulated that the thermal death point of the mycelium was approximately the same as for the spores. O'Brien (1902) found that the mycelium of *Botrytis vulgaris* was killed at 50° C. for exposures of 30 and 60 minutes.

Some attempts were made to determine the ability of the fungus to withstand low temperatures by the following method: Tubes of sterile distilled water were poured upon agar slant cultures well covered with spores, then poured back into the original tubes thus giving dense spore suspensions. Other agar slant cultures showing a profuse growth of spores, and some showing no spores but copious mycelial growth were placed, along with the water-spore suspensions mentioned above, in a receptacle, then carefully surrounded with cracked ice and salt. At the outset, the thermometer, also packed in the ice and salt mixture, registered below 0° C., and the water was frozen solid. These tubes remained in the ice box for three days when cultures were made from them. Transfers from all of the tubes gave positive results. In this connection it is also interesting to note that Brooks and Cooley (1917) found that *Botrytis cinerea* when inoculated into apples grew at 0° C. This fungus also grew in cornmeal agar in petri dishes at 0° C.; and the spores germinated in cornmeal agar at 0° C. in one month. According to Hoffman (1860) the spores of *Botrytis vulgaris* germinated very near the freezing point and did not lose their vitality at temperatures below freezing; however, such temperatures were fatal to germinating spores.

The results of the study of the temperature limits for the life and growth of *Botrytis allii* on culture media show that: (1) The high thermal death point of 50° C. (122° F.) will permit this fungus to survive in the field in any of the onion growing states; (2) the cultures, spores, or mycelium are not killed by freezing — or low temperatures. It is, therefore, reasonable to conclude that the fungus may readily live over from year to year in the onion fields.

Relation to environmental factors.—Reaction to temperature:—The fungus evidently passes the period of dormancy in the soil at relatively low temperatures; therefore, the ability of the fungus to withstand cold is important. Trials bearing upon the reaction of the fungus to temperature were conducted as follows: Immediately after inoculation with the *Botrytis* spores, tubes of onion agar were placed in each of nine compartments of a home-made differential thermostat designed to give a wide range of temperatures. A compartment at one end of the apparatus contained about one liter of water which was renewed automatically and which was kept at the boiling point by an electric heater. At the other end of the row of compartments a larger space was filled with ice and salt. When kept carefully iced the variation in the range of temperature of each compartment was insignificant. The results of a single trial are as follows:

TABLE II. — GROWTH OF BOTRYTIS AT DIFFERENT TEMPERATURES ON ONION AGAR.

Compartment.	Temperature.	Growth at end of 10 days.
	<i>Deg. C.</i>	
1.....	8	Small amount of growth; no spore production.
2.....	12	Fair amount of growth; few spores produced.
3.....	20	Growth abundant; much fruiting.
4.....	24	Growth abundant; medium amount of fruiting.
5.....	26	Growth abundant; a few spores produced.
6.....	28	Growth medium; very few spores produced.
7.....	31	Growth slight; no spores produced.
8.....	40	Growth faint.
9.....	45	No growth perceptible.

From the results of these tests it is evident that a temperature of about 35° C. is unfavorable for the growth of the fungus even tho, as reported in a previous paragraph, the fungus may survive a temperature of 50° C. A slight amount of mycelial growth with

no spore production took place at 8° C. The optimum temperature was quite clearly shown to be approximately 20° C. That spore production took place only between the temperatures of 12° and 28° C. is rather interesting and significant in view of the recent work of Beauverie and Guilliermond (1903), who worked on the polymorphism of *Botrytis*. By controlling certain factors, mainly moisture and temperature, they produced a strain of *Botrytis* which did not form conidia. Coons (1916), who reviews the literature bearing upon the subject in a recent paper, has shown conclusively that the temperature limits for reproduction of the fungus pathogene studied by him are much narrower than those for growth.

Reaction to light:—Of all of the various tests made with cultures of the fungus upon various kinds of media and placed in light and darkness, with the same degree of aeration, no indications were noted that there was any marked response to either of these factors. Darkness appeared to favor slightly the formation of sclerotia. It was only when the fungus was grown upon a medium poor in nutrients that a slight response to light in the direction of increased production of conidia could be detected. This latter observation appears to be in accord with the results of Lendner (1896) who tested the effect of light upon *Botrytis* among other species of fungi. Reidemeister (1909) reports that with *Botrytis cinerea*, light favors the production of conidia while darkness favors the formation of sclerotia.

Reaction to aeration.—Of all the factors tested, the reaction of the fungus to aeration was most marked, it being sensitive to the oxygen supply. Cultures of the fungus in sterile flasks of liquid media, vegetable tissues, and bread soaked with various fruit juices, which were sealed by covering the cotton plug with paraffin, showed only a very scanty production of conidia in most cases, while unsealed flasks produced an abundant crop. The growth, which was very abundant in the sealed flasks, was mainly in the form of a close-growing, dense mat of white mycelium and a fairly abundant crop of sclerotia. The effect of aeration upon the formation of sclerotia and organs of attachment or appressoria was well illustrated in a series of tests in which tubes of onion agar inoculated with the onion *Botrytis* spores were poured in petri dishes which were then placed in deep battery jars kept moist by means of wet cotton. While no conidia were produced on these cultures, a very profuse

formation of organs of attachment and many sclerotia resulted. Rings of small sclerotia were formed in the petri dishes where the medium was thin due to the intentional tipping of some of them while they were cooling (Plate XI, fig. 1). All of these observations appear to agree with the conclusions of Reidemeister (1909) who investigated the conditions of sclerotium and sclerotial ring formation of *Botrytis cinerea* and demonstrated that increased transpiration favored sclerotium formation and retarded production of conidia.

All evidence pointed to the fact that under increased transpiration conidia were formed abundantly, but no sclerotia; while under conditions of very meager transpiration a generous crop of sclerotia, many appressoria, and no conidia were produced. With these facts in mind it was possible to explain the conditions found in the storage houses, where, in a very humid atmosphere the fungus developed mainly sclerotia, while a profuse formation of conidia resulted when the diseased bulbs were removed to the outside air or to the drier atmosphere of the laboratory. Large quantities of diseased bulbs, partially or completely covered with sclerotia, when removed from the storage houses to the dump heap were soon covered with a dense crop of conidia. These serve as a virile source of infection to the growing crop of onions.

Reaction to desiccation.—The length of time that the conidia may live was deemed very important. From dried cultures in test tubes on onion agar the conidia gave a rapid response to conditions favoring germination after 21 and 24 months. At the end of three years the conidia from these dried up cultures showed a very weak germination, and a very low percentage. The length of life of the mycelium in dried cultures was by no means as long as that of the conidia, it being approximately nine months to a year. The longevity of the sclerotia when dried was approximately double that of sclerotia kept in moist earth.

Reaction to moisture.—The fact that the diseased onion bulbs, when in a very damp storage house, showed a copious sclerotium production with but scanty conidium formation suggested moisture as an important factor. When bulbs bearing these sclerotia were transferred to another storage house with a drier atmosphere, other factors being approximately equal, a copious conidium production took place. These indications were substantiated by the results of moisture tests with culture media in tubes and flasks in which

excessive moisture was shown to delay conidium production somewhat and to facilitate sclerotium formation. To a series of 200 cc. flasks each containing 10 grams of sterile cornmeal there were added 10, 20, 30, 40, and 50 cc. of sterile water. These were inoculated with the onion *Botrytis* spores. In these flasks, as they stood, drying took place and brought about a range of conditions. The best condition for the production of conidia appeared to exist in the flask containing 20 cc. of water, while conidia matured slightly earlier in the flask containing 10 cc. of water. The most favorable conditions for sclerotium formation existed somewhere in the range of flasks containing between 20 cc. and 30 cc. of water and the 10 grams of cornmeal. Evidently, the range of moisture conditions which favor the formation of conidia and sclerotia is not a wide one.

Biochemical relations.—Relation to acids and alkalis.—A study of the reaction of some of the common media used in determining the cultural characters of this fungus indicated that the range of growth and production of conidia was a rather wide one. In order to determine the optimum acidity or alkalinity which favors growth and spore production the fungus was grown in flasks of prune and onion juice which were first neutralized and then reinforced with definite amounts of malic acid or sodium hydroxid. Definite amounts of the sterilized and neutralized plant juices were placed in flasks to which were added the required amounts of sterilized acid or alkali. The flasks were then inoculated with spores. Table III gives the results of a single test with prune juice. The titrations are expressed in terms of Fuller's scale, computed in terms of cubic centimeters of normal hydrochloric acid or potassium hydroxid in a liter by titrating 5 cc. with N/20 standards, using phenolphthalein as an indicator.

From these results it will be seen that this fungus produced a crop of conidia on some of the alkali series, the growth in these flasks having the typical smoke-gray color. On the acid series the growth was considerably lighter in color. The vegetative growth in the neutral flask was whiter in color than that in any of the other flasks. The rate of growth was very materially retarded in the concentrations greater than -20° and $+20^{\circ}$. While the range over which conidium production may take place is comparatively wide, the range of vegetative growth is surprisingly wide, being approximately -100° to $+100^{\circ}$ Fuller's scale. The results seem to indicate

that the strongest mycelial growth is associated with low acidity or alkalinity, while a comparatively high concentration of either favors conidium production. It seems apparent, however, that the solution used (prune juice) is rather rich in nutrients, especially sugars.

TABLE III.—THE EFFECT OF ACIDITY AND ALKALINITY OF THE MEDIUM UPON GROWTH AND CONIDIUM FORMATION OF *Botrytis Allii*.

Reaction, Fuller's scale.	GROWTH DURING EXPERIMENT: TIME, 1 MONTH.	
	Mycelium.	Conidia.
—300.....	None.....	
—200.....	None.....	
—100.....	Very little.....	
— 50.....	Small amount.....	Fruiting abundantly.
— 30.....	Small amount.....	Fruiting abundantly.
— 20.....	Medium amount; felt thin.....	Abundant conidia.
— 10.....	Fair growth.....	Fruiting moderately.
— 5.....	Good growth.....	Very few conidia.
— 0.....	Best felt of mycelium.....	Fruiting less than —30.
+ 5.....	Slightly better than —5.....	Fruiting much less than on alkali series.
+ 10.....	Slightly better than —10.....	Fruiting much less than on alkali series.
+ 20.....	Slightly better than —20.....	Fruiting much less than on alkali series.
+ 30.....	Medium amount.....	Fruiting much less than on alkali series.
+ 50.....	A little.....	Fruiting much less than on alkali series.
+100.....	Very little growth.....	Very few conidia.
+200.....	Scant.....	Very few conidia.
+300.....	None.....	

That this species of *Botrytis* is not especially sensitive to acids is in accord with the results obtained by Ward, who states (1888, p. 334) that with the lily *Botrytis* the degree of acidity tolerated may be considerable. On the other hand, Zschokke (1897) stated that *Botrytis cinerea* shuns sour fruits such as currants and sour apples. Under the heading of different media tested the writer gives some results from the use of sweet and sour apples. The acid and alkali tolerance of this fungus is remarkable. It must be a factor in the growth of an organism living on a highly nitrogenous substratum, where ammonia accumulates.

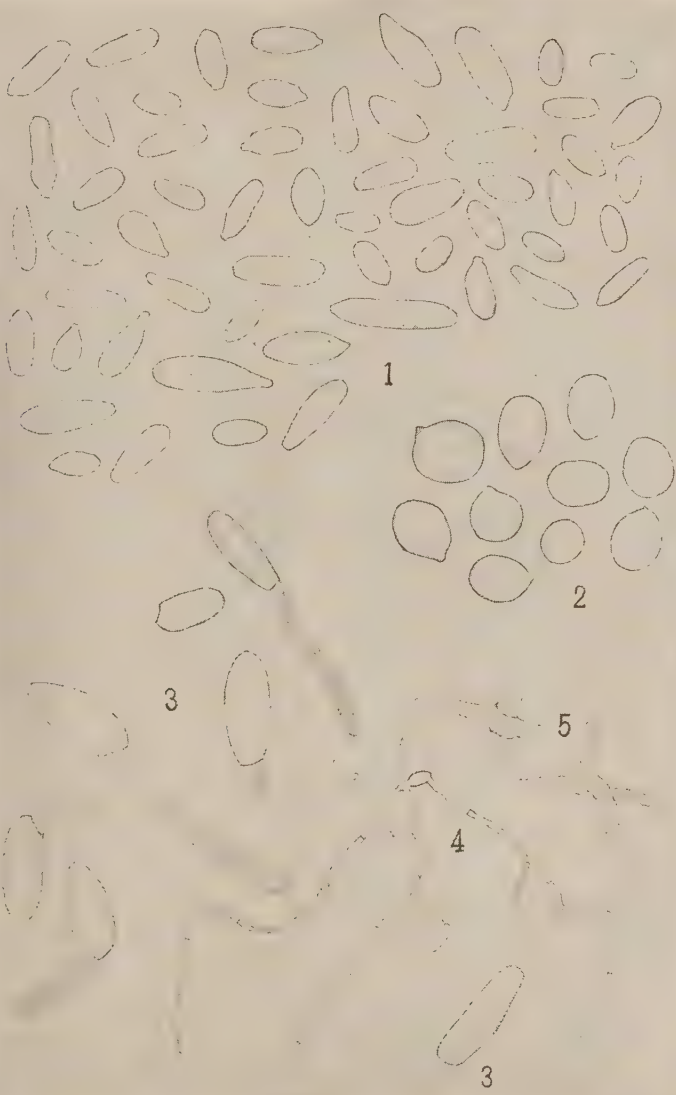


PLATE I.
(See back of Plate XI.)

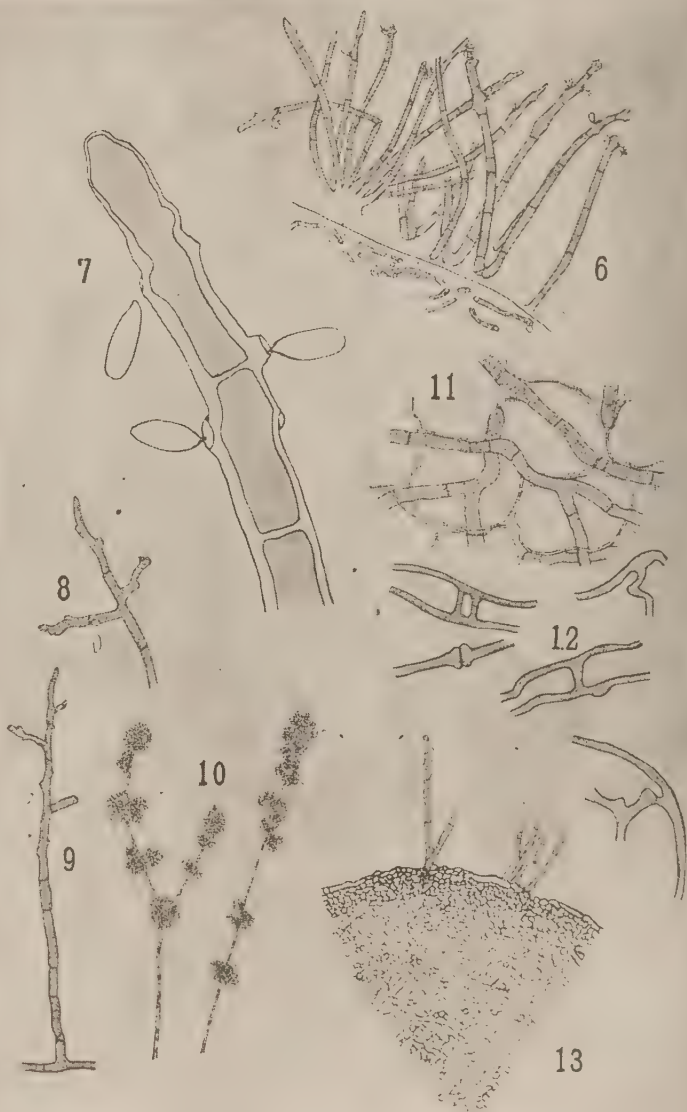


PLATE II.
(See back of Plate XI.)

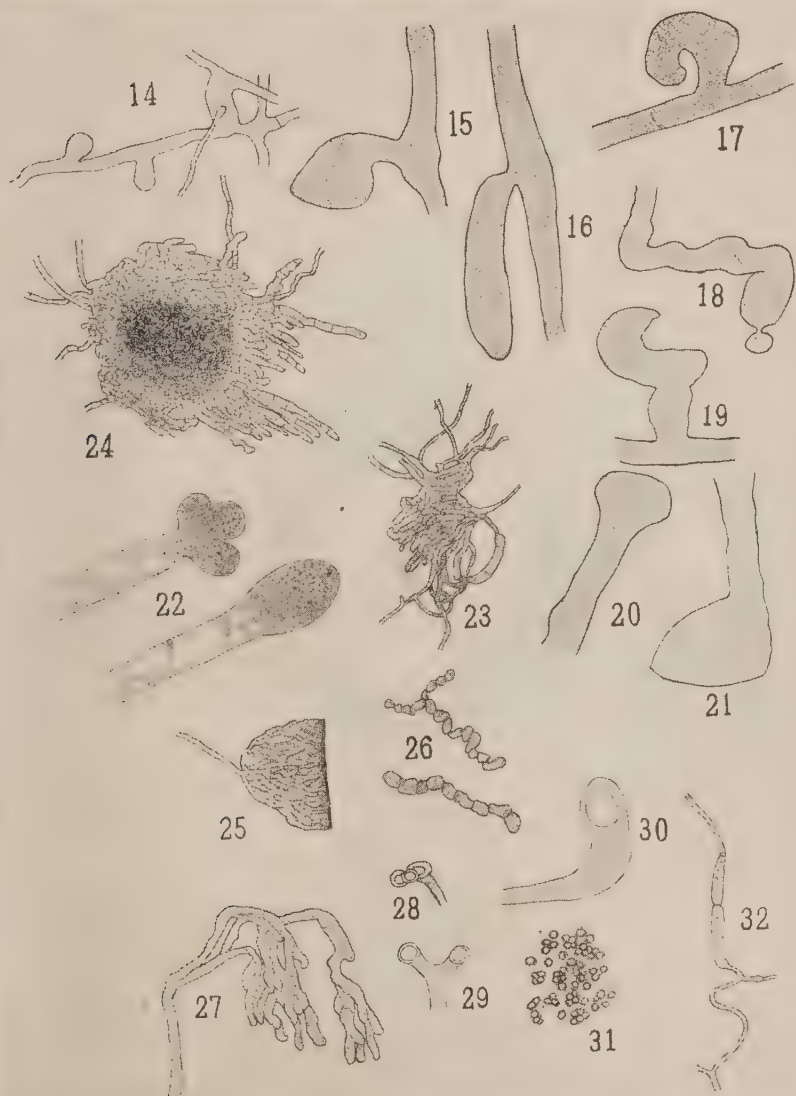


PLATE III.
(See back of Plate XI.)



PLATE IV.
(See back of Plate XI.)

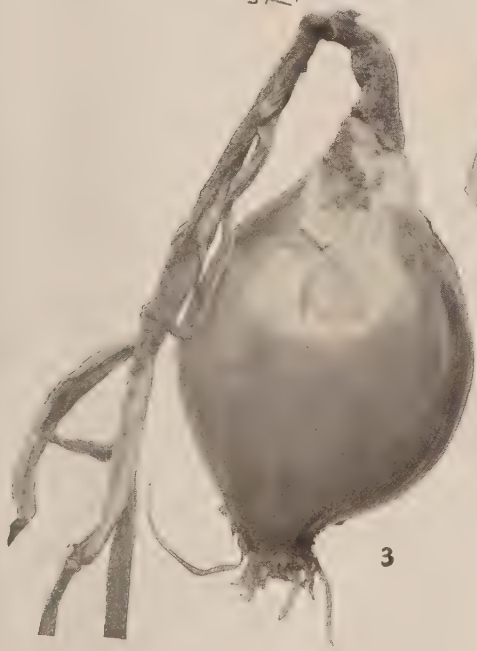
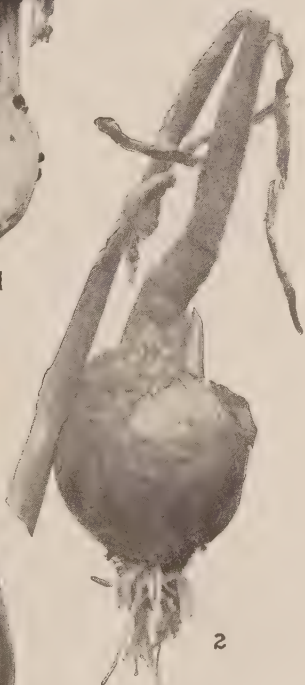
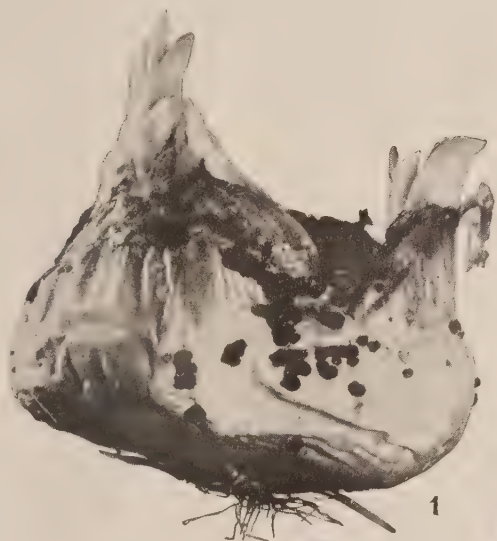


PLATE V.
(See back of Plate XI.)

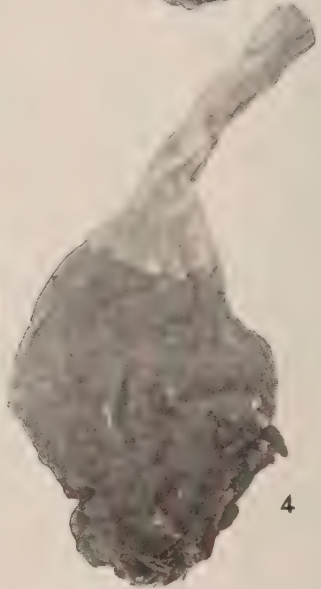
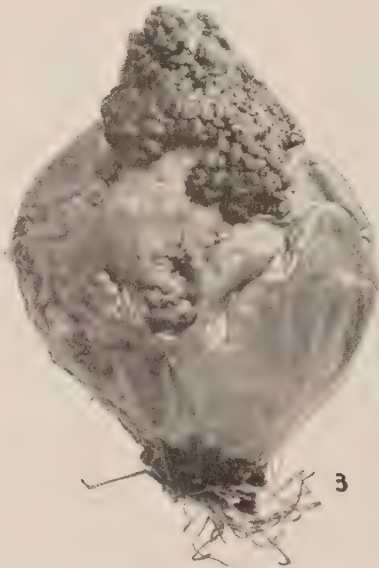
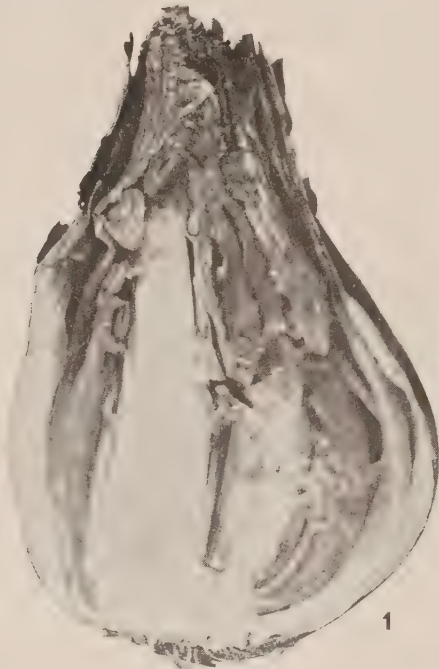


PLATE VI.
(See back of Plate XI.)

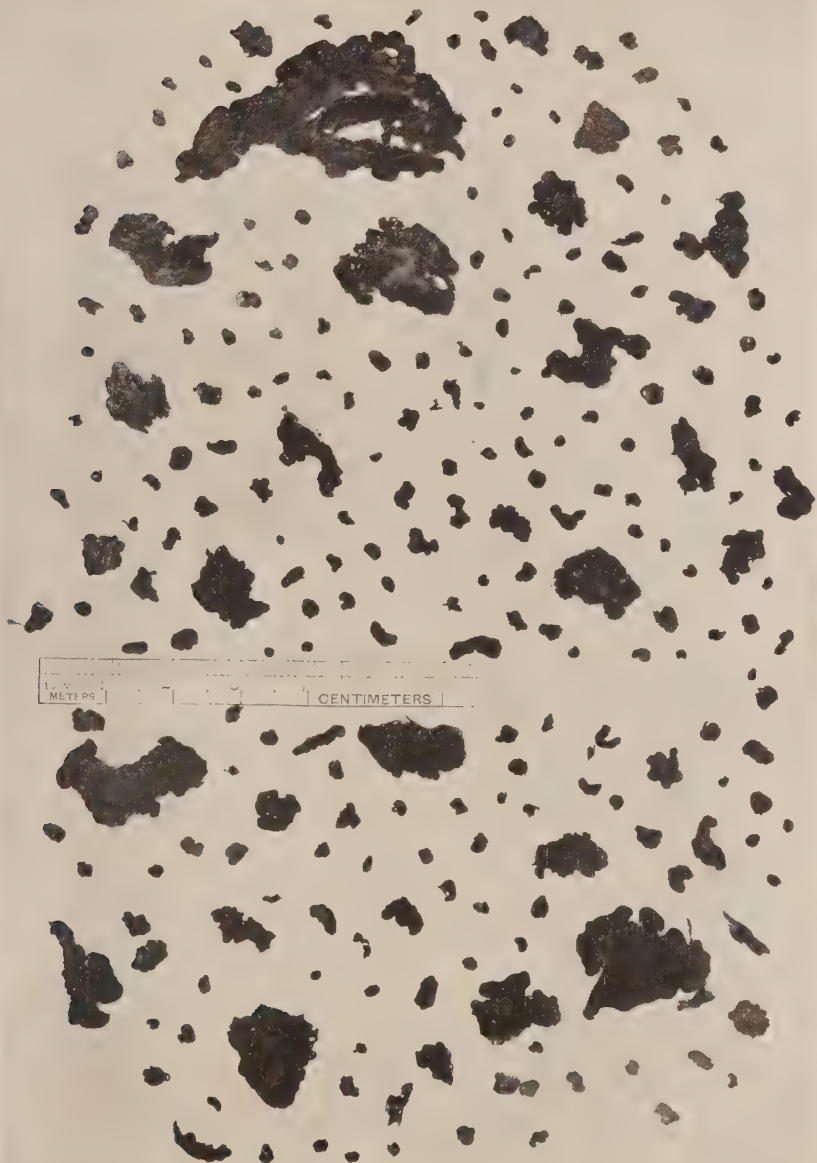


PLATE VII.
(See back of Plate XI.)



PLATE VIII.
(See back of Plate XI.)

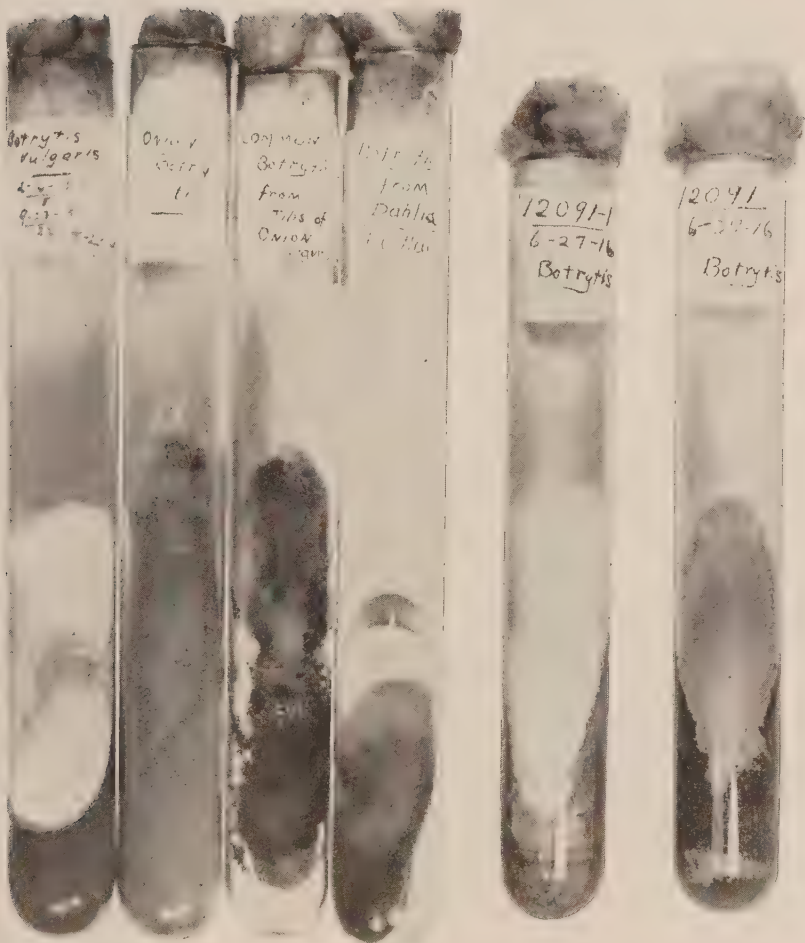
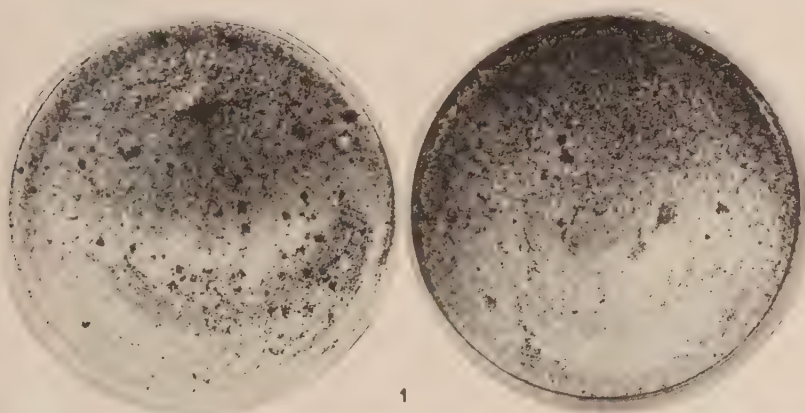


PLATE IX.
(See back of Plate XI.)



PLATE X.
(See back of Plate XI.)



1



2

PLATE XI.
(See back of Plate.)

EXPLANATION OF PLATES.

PLATE I.—

- Fig. 1.— Conidia of *Botrytis allii* n. sp.
- Fig. 2.— Conidia of *Botrytis cinerea* Pers.
- Fig. 3.— Germinating conidia of *Botrytis allii* n. sp.
- Fig. 4.— Germinating conidium after 48 hours; showing cross connection and method of branching.
- Fig. 5.— Cross connections in mycelium.

PLATE II.—

- Fig. 6.— *Botrytis* on onion bulb scale.
- Fig. 7.— Portion of conidiophore. (Much enlarged; camera lucida drawing.)
- Fig. 8.— Portion of tip of conidiophore from pure culture. Shows tendency toward more profuse branching in cultures.
- Fig. 9.— Typical conidiophore from onion bulb. (Outlined with camera lucida.)
- Fig. 10.— Conidiophores of onion *Botrytis*.
- Fig. 11.— *Botrytis* mycelium in onion bulb scale.
- Fig. 12.— Cross connections of mycelium in host tissue.
- Fig. 13.— Cross section of germinating sclerotium.

PLATE III.—

- Fig. 14.— Type of mycelium common in pure cultures.
- Figs. 15-22.— Types of structures on mycelium in leaf tissue. (Camera lucida drawing.)
- Figs. 23 and 24.— Developmental stages of the sclerotium.
- Fig. 25.— Side view of holdfasts.
- Fig. 26.— Type of heavy mycelium in pure cultures.
- Fig. 27.— Incipient stages of sclerotium formation from organs of attachment.
- Fig. 28.— Organs of attachment; view from beneath plate.
- Fig. 29.— Organs of attachment, more enlarged; from beneath plate.
- Fig. 30.— Appearance when tip of hypha touches glass.
- Fig. 31.— Bottom view of holdfasts on glass plate.
- Fig. 32.— Flattening of mycelium as it comes in contact with glass culture dish.

PLATE IV.—

- Figs. 33 and 34.— Typical budding, or sporulating filaments of onion *Botrytis* in media. (Camera lucida drawing.)

PLATE V.—

- Fig. 1.— Appearance of disease on white onions.
- Figs. 2 and 3.— Appearance of the disease on necks of plants.

PLATE VI.—

- Fig. 1.— Longisection thru diseased bulb.
- Fig. 2.— Infection thru bottom of bulbs.
- Fig. 3.— *Botrytis* sclerotia at necks of bulbs.
- Fig. 4.— Sclerotial crusts on onion bulb.

PLATE VII.— SCLEROTIA OF *Botrytis allii* n. sp.

PLATE VIII.—

- Fig. 1.— Two diseased and one healthy seed head.
- Fig. 2.— Seed head (blasted) attacked by the fungus.
- Fig. 3.— Effect of fumigation with formaldehyde gas on onions.

PLATE IX.— ONION BOTRYTIS CULTURES COMPARED WITH OTHER BOTRYTIS CULTURES.

PLATE X.—

- Fig. 1.— Germination of sclerotium by forming stalk-like bodies.
- Fig. 2.— Effect of fungal extract upon onion bulb scale.
- Fig. 3.— Onion bulb injected with spore suspension.

PLATE XI.—

- Fig. 1.— Sclerotial ring formation of *Botrytis* in petri dishes.
- Fig. 2.— A very desirable type of onion storage house.

LIFE HISTORY STUDIES.

Primary infection of bulbs in the field.—From the early studies of the disease the writer was convinced that the mycelium was present in the bulbs when they were placed in storage where the disease developed with more or less rapidity depending upon the storage conditions. It has been found that approximately 80 per ct. of the diseased bulbs in certain instances showed the presence of the fungus at the neck, the remaining 20 per ct. showing the fungus upon the sides and bottoms of the bulbs. Examinations have shown that the fungus attacks the plants thru the necks and frequently thru the yellowing leaves where it works its way down into the neck of the bulb, remaining as a few mycelial threads or even as a mycelial mat between the outer three or four scales. Moreover, infection experiments, previously discussed, were made at the outset of the work, which yielded facts in accord with the above idea that infection takes place on the neck and passes to the bulb. In one plat of white onions under observation near East Lansing, Michigan, the neck-rot fungus was found fruiting abundantly on the necks and bulbs of the dying onions in October. A few weeks later when the bulbs were examined in storage they were found to be fairly well covered with the black sclerotia of the fungus.

That infection may also take place thru the bottom and roots of the bulbs is clearly evident. Here, the bulbs show abundant mycelium and masses of sclerotia at the bottom (Plate VI, fig. 2). As a rule the bulbs which show bottom infection are considerably under normal size and do not show the normal amount of roots attached when examined in storage. These bulbs invariably do not show the fungus mycelium in the upper parts of the bulbs. Experiments gave conclusive evidence that the fungus may pass from the soil to the bulbs and in such a manner as to escape the notice of the casual observer. It is not an uncommon occurrence to find onions which show the fungus at both the neck and bottom of the bulb. Infection may also take place thru wounds in the bulbs, and also thru onion-maggot injuries.

Infection of bulbs at harvest time.—When wet weather occurs during the harvesting and curing period, while the bulbs are drying in the field, a considerable amount of infection may take place thru the dying and drying leaves, especially when the bulbs are curing in the windrows. After being stored the neck rotting begins.

The writer visited a large storage house in central New York where 12,000 crates of onions were stored. According to an estimate made by the owner about one bushel out of every seven from certain lots was diseased. An examination of these diseased bulbs revealed a very striking condition in that nearly every bulb showed side infections, i. e., the disease showed at the sides of the bulbs and not at the necks or bottoms. This at once suggested a bruise or injury of some kind. Upon inquiry it was learned that an onion-topping machine had been used upon the greater part of this lot of onions. The bruising of the sides of the onions by the topping machine was the only explanation that could be offered for this condition of infection. The owner of the storage house had come to look with disfavor upon the topper because he had learned that machine-topped onions did not keep quite as well in certain seasons as hand-topped stock. He claimed that the topping machine bruised the sides of the large, sound bulbs, or as he expressed it: "it gives them quite a rap." A similar condition as regards side infections following the use of the onion-topping machine was encountered in one of the Allegan County, Michigan, onion regions. Here, the storage house operators claimed "that the bulbs were bruised by the machine and consequently rotted." On this account some growers object to the use of certain types of onion-topping machines because of the serious bruising of the bulbs. The writer believes that it will be found, thru a series of experiments which are now under way, that machine topping does not seriously injure the keeping quality of the onion if the stock is well cured. Very seldom does one find a bulb which has been injured during the process of topping when the work has been done by hand with shears. A certain amount of infection will take place thru wounds made by forks or shovels used in placing the onions in crates either before or after topping.

Secondary infection of bulbs in storage.— But very little secondary infection is believed to take place in storage, since the "contacts" (those healthy bulbs lying against diseased bulbs) very seldom become diseased unless the storage house is very damp and the dry outer skins become soft and full of water. The dry outer scales are usually the last to rot and the disease does not seem to spread from onion to onion thru the dry skins. Infection experiments which have been previously discussed show that the spores of the fungus

when placed upon the dry onion scales, even under a bell jar, are unable to bring about infection.

Overwintering and dissemination of the fungus in the field.— From the outset of the investigation it was believed that the fungus causing the neck rot of onions was present in the soil and overwintered in the field. This idea was confirmed early in April, 1915, when the fungus was found fruiting upon some pieces of onion leaves in an onion field in central New York. Less than one-half mile from this spot there were several hundred crates of neck-rotted onions scattered in an old orchard less than forty rods from an onion seed field in which the fungus was found to be destructive to the seed crop later in the season. Altho the refuse onions had been dumped in the orchard early in December it was not difficult to find hundreds of bulbs which were covered with a profuse crop of conidia on this date. Again, on April 28 and on May 3, 1916, while going over an onion field in Michigan the writer found abundant spores being produced on refuse onion tops which had remained in the field all winter. Sclerotia were also found on some "scallions" or "thicknecks" (onions which develop no bulb, but have a long thick neck) which were discarded in the field at the time of harvesting. In both of these instances the disease was known to have been present and destructive in the fields the previous season.

As early as the middle of June the writer has observed the fungus attacking the stalks of the seed crop onions. Later in the season and even until late fall it was found upon onion bulbs and onion refuse left in the field, and again in the spring in the same field the fungus was found in its *Botrytis* form upon the old onion tops and necks left after the crop onions were topped the previous season.

It was found to be comparatively easy to isolate the onion *Botrytis* from the soil by using the method described by Jensen (1912). This author also gives a list (pp. 489–491) of seven species of *Botrytis* isolated from the soil.

Cultures made from the above-mentioned lots of material, supplemented by field observations and some experiments reported in this paper prove beyond doubt that the fungus may pass the winter on the diseased tops and necks of refuse onions as well as in the soil. The growing crop and also the seed crop may become infected from either of these sources. However, it is believed

that the dumping of diseased refuse onions near seed fields becomes the most virulent source of infection for the onion flowers.

Dissemination in seed fields thru transplanting diseased bulbs.—Attention has already been called to the fact that the bulbs ("mother bulbs") used for the production of onion seed, when diseased, have a marked effect upon the amount of seed produced. There is great danger of transferring the disease to the seed fields by means of the infected bulbs. From the seed fields it readily spreads to the growing crop onions. Diseased bulbs usually produce a few small, erect, dark green leaves which die down very early.

Occurrence of the fungus spores on onion seed.—Since the fungus is seriously destructive on the flowers in certain seasons, a study was made of the possibility of the fungus being disseminated by means of spores adhering to the seeds. A quantity of seed was secured from a seed field in which the neck-rot fungus was destructively prevalent during the season of 1915. Quantities of this seed were then washed in distilled water and the washings poured into a centrifuge tube. After centrifuging at 2000 revolutions per minute for five minutes the clear liquid was decanted off and the sediment examined under the microscope for the fungus spores. In addition to a large number of spores of the *Botrytis* neck-rot fungus, there were found spores of the smut fungus (*Urocystis cepulae*), also spores of species of *Cladosporium*, *Macrosporium*, *Fusarium* and *Rhizopus*. In one instance the writer was fortunate in securing a small quantity of seed from the 1914 crop which was produced on the same field as the 1915 seed crop. The washings from this seed also showed the spores of the neck-rot fungus to be present and in addition spores of a species of *Rhizopus*.

RELATION OF PARASITE TO HOST¹

At the beginning of the investigation it was noted that a peculiar relation existed between the host and parasite. A macroscopical examination of the leaves, necks, and bulb scales of the host, when attacked by the fungus, reveals a discoloration of the tissues, and very often a boiled or water-soaked appearance somewhat in advance of a point where the fungus may be seen fruiting.

¹ An abstract of this section was read before the Botanical Section of the Michigan Academy of Science in its Twenty-first Annual Meeting at Ann Arbor, Michigan, March, 1916.

HISTORICAL REVIEW.

Kühn (1858) in speaking of the penetration of the host tissue by fungous hyphae mentions it as a process of boring thru ("Durchbohrung").

De Bary (1863, 1866, and 1884) mentions the process of the penetration of the host by fungi, but states that he had no knowledge of the forces causing this boring into the tissue. This author (1884) working with *Sclerotinia libertiana*, appears to be the first to report that a substance was produced which discolored the host tissue, plasmolyzed the cells, and later killed them. De Bary was able to isolate this thermolabile substance and ascribed its cell-wall-dissolving action to an enzym, altho in one place he suggested the possibility of the toxic action being due to oxalates which he found encrusting the mycelium. It appears that he later changed his ideas in regard to the action of the oxalates since solutions of oxalic acid did not give such markedly destructive effects.

Ward (1888) in a searching investigation of a lily disease caused by a Botrytis found that the tips of the fungus hyphae secreted a substance which "has the property of swelling and dissolving the cellulose cell walls." This author ascribed this action to a soluble ferment.

Kissling (1889), using methods similar to those employed by Ward, found that *Botrytis cinerea* formed an enzymic poison which kills the invaded tissue of the host. Apparently, his work confirms that of De Bary.

Kean (1890) also noted the "glairy fluid" exuding from the tips of hyphae of the same Botrytis growing upon *Lilium* sp. as was noted by Ward. This author was also able to demonstrate the softening and decomposition of pieces of healthy sweet potato tissue by the expressed and filtered juice from a rotting sweet potato which had been attacked by *Rhizopus nigricans*. He then obtained a precipitate from this filtered juice by the usual alcoholic precipitation, which carried with it the active principle of the extract. Kean was also able to get similar results and a similar substance from Ward's lily Botrytis and from a Botrytis growing upon *Sedum telephium*.

Nordhausen (1899) working with *Botrytis cinerea* found an enzym present which behaved similarly to that of De Bary's *Sclerotinia*.

Behrens (1898) working with *Botrytis cinerea* among other fungi, found in addition to a pectin-dissolving substance, a thermostable

toxic body which had a marked action upon the host tissue. This author concludes that *Botrytis* is capable of dissolving cellulose.

Smith (1902) from his work with *Botrytis cinerea* reports dissolving action by this fungus upon the middle lamella. This action he found to occur even after the fungal extract had been boiled. This author believed the dissolving of the cell wall to be due to oxalic acid, but does not give his methods for determining the presence of the acid.

Schellenberg (1908) studied the action of various fungi upon hemicelluloses and concluded that the penetrating and dissolving power of *Botrytis cinerea* is due to its ability to dissolve hemicelluloses. He believed the middle lamella to be composed of hemicelluloses.

Jones (1909) in his painstaking work with the cytolytic enzymes produced by *Bacillus carotovorus* and other soft-rot organisms (forty-five from different sources) shows conclusively that a middle-lamella-dissolving enzyme is produced. This author gives a complete resumé of the literature dealing with phyto-pathogenic bacteria and their relation to the host tissue.

Bruschi (1912) grew *Monilia cinerea* in a medium containing the flesh of the plum, and after forty-eight hours noted that all of the cells were separated one from another, therefore he concludes that the enzyme pectinase was produced by the fungus. He was unable, however, to isolate a cellulose-dissolving enzyme.

Peltier strongly emphasizes the two-fold aspect of the disintegration of the host tissue by a *Botrytis* appearing as a parasite on peppers. This author believes that some substance is secreted by the fungus which kills the tissue in advance of the mycelium.

Brown (1915, 1916), whose work will be reviewed below, appears to be the first to attempt to obtain the active principle from the young fungal elements of a *Botrytis*. This investigator made a powerful extract from the germ tubes and showed that the only active substance present in the fungal extract is an enzyme which appears to be responsible for the two-fold action of the plant tissue.

Valleau (1915), working with *Penicillium expansum*, performed some experiments which strongly indicate that that fungus secretes a very active middle-lamella-dissolving enzyme, pectinase. This author was unable to extract the enzyme pectinase from an extract of the fungus *Sclerotinia cinerea*, or to demonstrate its presence in rotting fruits.

Because of the peculiar mode of action and method of infection of the onion fungus a study was made of some of the striking features of its method of parasitic attack upon the host.

PATHOLOGICAL HISTOLOGY.

A microscopic examination of sections of a large number of the diseased bulbs from storage houses showed that invariably the host tissue was killed somewhat in advance of the tips of the hyphae of the fungus. This was very easily shown by cutting small blocks containing both healthy and diseased tissue from the affected bulbs. These blocks were plunged into 95 per ct. alcohol for slightly less than an hour for most of the blocks. Free-hand sections containing both diseased and healthy tissue were stained in eosin for a few minutes, then decolorized in acid alcohol for an instant. These sections show the injured cells to be considerably darker than the healthy cells. The fungus is differentiated from the host cells which remain unstained. The diseased areas show plainly that there is a toxic or lethal action, or both, in advance of the hyphae. The cells are discolored, the degree of the discoloration being a possible index of the severity of the toxic action. Following discoloration, the cells are plasmolyzed, the contents become granular and later the cell walls collapse, this being regarded as a lethal action. It seems that the middle lamella is readily dissolved by the action of the fungus which later sends its hyphae between the cells, directly thru the disorganized cell walls, or across the intercellular spaces and very often directly from one cell into the next (Plate II, fig. 11). The host tissue may be found as a disorganized mush of a firm consistency filled with the fungus mycelium; or the cells, in some of which the distorted protoplast may still be seen, may appear to be held in place by the mycelium. From this effect on the host it would appear that a middle-lamella-dissolving enzym is produced. A consideration of a few typical cases will serve to make these points clear. Figure 3, Plate X, shows one of a number of sound bulbs which were injected with a spore suspension of the onion *Botrytis* spores in distilled water, using a hypodermic syringe. The point of inoculation is at A. From this point the heavy *Botrytis* mycelium was found extending out in all directions and gradually decreasing to the point B where none was to be found. From B to the margin or border of the diseased area the tissue was discolored, and the cells were plasmolyzed and loosened.

A longisection thru a diseased bulb is shown in Figure 1, Plate VI. between the scales near the neck and extending down nearly to the center of the bulb one can see the mycelium woven together in the form of a dense web or crust. All of the scale tissue below, altho no mycelium was found to be present, was discolored and the cells were broken down. The sprout in the center has been attacked at the upper end where it is in contact with the diseased scales and shows some discolored and softened tissue.

Stevens (1916) in a recent paper describes an almost identical condition as found from a study of the pathological histology of strawberries affected by *Botrytis* sp. This author concludes that the fungus is evidently capable of not only dissolving the middle lamella but of penetrating the cells themselves.

CELL WALLS OF THE HOST.

Since the fungus showed a very marked action upon the host-plant cell walls a brief study was made of the tissue of the necks and bulbs.

Tests for cellulose were positive in all cases, the best reactions being secured with cuprammonia or Schweizer's reagent in which the cellulose is soluble. A concentrated chloridid of zinc solution, acting directly upon the sections and giving them a violet color, was also successfully used.

The middle lamellae of the host cell membranes were shown to consist of pectic acid, or its insoluble salts, by treating thin sections for twenty-four hours with a mixture composed of one part hydrochloric acid and four parts 95 per ct. alcohol. At the end of this time they were washed with water, then treated with a 10 per ct. solution of ammonia for a short time when the sections could be easily separated into their constituent cells by gentle pressure. When these sections were stained with methylene blue for a few minutes after treatment with the acid alcohol the middle lamellae stained very deeply. The results of these and other tests applied agree with the statements of Mangin (1892, 1893) that pectose is very pronounced in the cell walls of young tissue, but in older tissue calcium pectate comprises the greater part of the middle lamella.

PRODUCTION OF OXALIC ACID BY THE FUNGUS.

Preliminary to a study of the enzymes produced by the fungus it seemed necessary to make a study of the possibility of oxalic acid being the toxic substance produced.

De Bary (1886), in his study of the fungus *Sclerotinia libertiana*, reported that the hyphae were often coated with crystals of oxalates. He found as high as 0.39 per ct. oxalic acid in one sample of his fungus. It appears that this author discounted the possibility of oxalic acid being the toxic substance since he was unable to secure the same effect upon plant tissue with solutions of oxalic acid as was obtained with the fungus or an extract of the fungus cultures. De Bary was unable to demonstrate any free oxalic acid.

Ward (1888, p. 336) reports the presence of minute crystals upon some of the conidiophores in his *Botrytis* cultures upon raisin extract.

Wehmer (1891), in a valuable series of articles reporting his work upon the production of oxalic acid by a number of fungi, points out that the fungi studied vary as to their ability to produce this acid, depending upon their metabolism as influenced by different factors which he gives. He holds that oxalic acid is a type of excretion.

Nordhausen (1899), working with a *Botrytis*, permitted the hyphae of this fungus to grow thru pieces of the epidermis of *Allium* which had been previously stained with congo red. The absence of a blue acid dye around the penetrating hyphae was taken as evidence of the failure of the fungus to excrete an acid, at least one as strong as oxalic acid.

Smith (1902), working with *Botrytis cinerea*, stated that he found over 20 per ct. oxalic acid in the extracts of mycelium from old cultures. This author was inclined to believe that oxalic acid is responsible for part of the action upon plant tissues. He was unable to detect any difference between the action of boiled and unboiled mycelial extracts upon petioles of lettuce leaves. Working with solutions of oxalic acid he reported a bleaching effect from the acid, which was noticeable to the naked eye.

Peltier (1912), in his work with a *Botrytis*, was unable to detect any difference between the boiled and unboiled extracts. Applying several delicate tests which, however, he does not outline, he was unable to detect the presence of oxalic acid. This worker also

tested the action of different concentrations of oxalic acid upon petioles of lettuce and found it to be somewhat like that of the mycelial extract, except that a bleaching of the tissues took place. He found this to be true of the organic acids and therefore concludes from his experiments that it is not oxalic acid, but may be some undetermined organic acid.

Cooley (1914), who gives his method of analysis, found oxalic acid to be produced in appreciable amounts by *Sclerotinia libertiana* when cultured on certain fruits.

Valleau (1915) studied the middle-lamella solvents concerned with the resistance of plums to brown-rot and reports some very interesting results with oxalic acid. He immersed blocks of tissue from various species of plants, among them onion and potato, in 0.015, 0.062, and 0.125 per ct. solutions of oxalic acid for stated periods of time. The various tissues showed varying degrees of resistance to the action of the acid, but all those affected showed that the softening was due to the solution of the middle lamella. The onion tissue softened in the 0.125 per ct. solution, while the potato did not soften even in a 0.25 per ct. solution. Valleau noted a marked bleaching effect produced by the acid and concludes from his experiments that oxalic acid "is not the sole toxic substance produced."

Brown (1916) found no evidence, at the end of twelve hours, of the action of a N/40 solution of oxalic acid or of N/20 potassium oxalate when placed on leaves of bean, or the petals of rose. He found, however, that the fungus (*Botrytis cinerea*) excreted an oxalate into the "infection drop," but that M/800 represented the upper limit of concentration. This author states that infection by the fungus could take place even tho the presence of a soluble oxalate was excluded.

Oxalate crystals in media.—While examining the medium directly beneath some cultures of the onion *Botrytis* growing on lettuce-leaf agar plus peptone (1 per ct.), the writer found a large number of crystals distributed thruout the substratum. These tetragonal pyramids had every appearance of calcium oxalate crystals. When treated with concentrated hydrochloric acid they dissolved slowly. At first it was believed that these were present only in the agar cultures containing peptone since Emmerling (1903), from his study of oxalic acid formation by certain fungi, stated that abundant

production of oxalic acid takes place on substances containing peptone. A further examination of the substratum from cultures of the onion *Botrytis* and ten other strains or species of *Botrytis* from as many hosts, and growing upon onion agar, showed the presence of these crystals. Examinations of lettuce-leaf agar cultures to which no peptone was added showed these crystals to be present. The cultures examined varied in age from 30 to 60 days. When carefully examined microscopically and tested microchemically these crystals appeared to possess all the properties and characteristics ascribed to crystals of calcium oxalate by Zimmermann (1893), Tunmann (1913) and Molisch (1913). Examinations made by Mr. O. B. Winter, Assistant Chemist of the Michigan Experiment Station, using the polarizing microscope, showed them to be of the monoclinic or tetragonal system. Hence, his observations confirmed those of the writer, since calcium oxalate possesses just such properties.

Oxalic acid in the diseased tissues.—In order to determine whether any free oxalic acid could be detected in the diseased, decaying onion bulbs, microchemical tests were made using calcium nitrate which showed no crystals of calcium oxalate to be formed. Other microchemical tests for oxalic acid as given by Zimmermann, Tunmann, and Molisch gave negative results.

Oxalates in the diseased tissues.—To ascertain whether any oxalates were produced by the fungus upon its host, chemical analyses were made of diseased and healthy onion bulbs. The analytical method used by Wehmer (1891) was employed. The soft, decayed tissue of onion bulbs overrun by the onion *Botrytis* was carefully cut away from healthy tissue. This material was then digested in cold hydrochloric acid, using in each lot 100 grams of the diseased onion tissue to 150 cc. of the acid. Controls were prepared by using 100 grams of healthy onion bulb scales sliced into 150 cc. of the acid. These lots were allowed to digest for 24 hours when the analyses were made.¹ The oxalic acid or its soluble oxalate was precipitated out as a calcium salt, which was filtered off, dried to constant weight and weighed. Results as follows:

Healthy onion tissue showed.....	0.0050 per ct. oxalic acid
Diseased onion tissue showed.....	0.0098 per ct. “ “

¹The determinations for oxalic acid were made by Mr. O. B. Winter, Assistant Chemist of the Michigan Experiment Station, to whom acknowledgments are due.

The analyses of liquid culture media yielded some very interesting results. Two hundred grams of clean onion bulbs were added to one liter of distilled water. This was then autoclaved for twenty minutes at fifteen pounds pressure when the juice was pressed out and 500 cc. placed in each liter Erlenmeyer flask and again autoclaved for 15 minutes at 15 pounds pressure. Some of the flasks prepared in this manner were inoculated with the onion Botrytis spores, the remainder was retained as checks. The cultures were 56 days old when the analyses were made. The liquid from beneath the fungous crusts was filtered off and the determinations made exactly as indicated above. The results are as follows:

Autoclaved onion juice, uninoculated. 0.0040 per ct. oxalic acid
Autoclaved onion juice, from beneath

growth. 0.0115 per ct. " "

The results of the determinations show that the onion Botrytis, when grown upon liquid media or upon onion bulbs, produces appreciable amounts of oxalic acid.

Titration of the liquid media.—At the time the determinations for oxalic acid were made, 5 cc. portions of the liquid from beneath the cultures were removed and added to 45 cc. of distilled water. This was then titrated with N/20 NaOH using phenolphthalein as an indicator. It required less than 0.1 cc. of the N/20 NaOH to neutralize the free acid in the juice; in fact, one drop of the N/20 alkali brought a distinct pink color. The juice was recorded as being +1° acid on Fuller's Scale. This would seem to indicate that there was but little free acid present.

Acidity of sound and diseased onions.—In the hope that the determination of the acidity of the diseased and sound onion bulbs would prove of value the following tests were made: Thirty-gram samples of the sound and diseased onion tissue were titrated with N/1 NaOH. The sound tissue was taken from several large healthy onions. The diseased tissue was taken from a number of onion bulbs attacked by the onion Botrytis. These lots of tissue were ground to a pulp in a mortar and then washed out with a large volume of distilled water into a large evaporating dish. The pulp and the water were thoroly stirred after which the titrations were made with N/1 NaOH using phenolphthalein as an indicator. The results of the titrations given in terms of cubic centimeters of normal alkali required to neutralize the acid in 30 grams of pulp are as follows:

30	grams of sound onion tissue required	1.2 cc. N/1 NaOH.
30	" " diseased " " " "	1.6 " "

In another series of tests at an earlier date the 30 grams of sound tissue required 1.3 cc. of normal alkali to neutralize the acid present.

From the results of these titrations it seems safe to conclude that the acidity of the tissue slightly increases following the attacks of the fungus. It is to be noted, however, that the bulk of sound tissue taken for titration is larger than the bulk of diseased tissue, and that the sound tissue seems to contain more juice than the diseased tissue.

Action of oxalic acid solutions upon plant tissue.—In view of the somewhat conflicting results concerning the action of oxalic acid upon various plant tissues and to study the possibility of this acid being a factor in the toxic action of the fungus studied, the following experiments were carried out: Various concentrations of oxalic acid were prepared using Kahlbaum's pure chemical. The concentrations in four cases were the same as those found in the samples analyzed and reported on pages 419 and 420. The action of the following concentrations was tested: 0.2000, 0.1260, 0.0115, 0.0098, 0.0050, and 0.0040 per ct. Pieces of onion-bulb scale, and potato-tuber tissue of the same thickness, namely 0.5 mm. were placed in these solutions and in beakers of distilled water for controls. No appreciable changes in any of the tissues could be detected until about six hours had passed when it was apparent to the naked eye that the potato-tissue discs in the four higher concentrations were bleached out whiter. No changes could be detected in the 0.0050 and 0.0040 per ct. solutions, except that the tissue discs were not as turgid as those in the water controls. The onion tissue did not show any noticeable changes. At this time the potato discs from the 0.2000 per ct. solution only, when compared under the microscope with the discs from the water, showed what appeared to be a reduction in size of the starch grains. No change in the cell walls could be detected.

In twenty-four hours the potato discs in the 0.2000 and the 0.126 per ct. solutions showed almost complete loss of coherence, that is to say, all of the cells were easily pulled or pressed apart. The cells floated free and rounded off, but the walls were apparently unaltered. The potato discs from the water controls were turgid, brittle and pulled apart with a cracking, splitting sound. All of the potato discs in all of the acid solutions showed a change in the starch grains,

i. e., these appeared to be smaller and present in larger number. The discs were denser when viewed under the microscope and compared with those from the water controls, apparently due to the change in the starch grains. At the end of the twenty-four hours the onion discs in only the 0.2000 and the 0.126 per ct. solutions showed a slight loss of coherence. None of the cells floated free as was true of the potato discs. No change could be detected in any of the other solutions except that all showed a bleaching or whitening effect upon the tissue.

At the end of forty-eight hours the potato discs in the 0.2000 and 0.126 per ct. solutions fell apart very easily, the cells appearing free with the walls intact. The discs in the 0.0115 per ct. solution showed a very slight loss of coherence, i. e., the tissue pulled apart, or was very slightly more easily torn than the tissue discs in the water controls. The potato discs in the 0.0098 per ct. or lower concentrations, aside from the whitened appearance, were in no way different from the discs in the water controls which retained their clear color and their turgidity. The onion discs from the 0.2000 and 0.126 per ct. solutions at the end of the forty-eight hour period were quite easily pulled apart, but they did not seem to have lost coherence of tissue to as great an extent as the potato discs. The onion tissue in the 0.0115 per ct. solution had unmistakably lost a very slight amount of tissue coherence, but the discs in none of the concentrations below 0.0115 per ct. showed any change except the bleaching effect which was evident in all of the concentrations. The discs in all of the solutions were flaccid, i. e., they were not as turgid as those in the water controls.

Plainly, at the end of forty-eight hours certain concentrations of the oxalic acid solution caused a solution or softening of the middle lamellae of the cell walls, the remainder of the cell walls remaining intact. All of the concentrations used brought about a bleaching or whitening of the tissues exposed.

The results from these and similar tests agree in many particulars with those secured by the investigators whose work has been reviewed above. All are agreed that oxalic acid solutions caused a bleaching of the tissues exposed. This was also true of other acids used. Valteau (1915) failed to get softening of potato tissue in a 0.25 per ct. solution. He stated, however that he used small blocks of the tissue. This author reported a slight softening of the onion tissue

in a 0.125 per ct. solution of oxalic acid. This compares closely with the writer's observations.

In the case of all of the tissue discs exposed to the action of oxalic and other acids, and examined microscopically by the writer, the softening of the tissue appeared to be due to a solution of the middle lamella. At all events, with the possible exception of such solution of the middle lamella, the writer is unable to find, in the action of the oxalic acid, any of the pathological changes taking place when the tissues are acted upon by the fungus itself. Even tho appreciable amounts of oxalic acid are formed by the fungus it cannot be regarded as the toxic substance secreted by the fungus. Furthermore, it is believed by the writer that it plays no appreciable part in the disintegration of the host tissue by the actively advancing mycelium.

PRODUCTION OF AN ENZYM BY THE FUNGUS.

Action of extracts from old mycelium.—Ward, Kean, Kissling, Smith, Nordhausen and Peltier made extracts from old *Botrytis mycelium* and noted its effect upon various plant tissues. Ward (1888), in studying the action of the lily *Botrytis*, made flask cultures of the fungus on prune juice. The mycelial crusts were removed at the end of three weeks and crushed in a mortar with a little distilled water. The liquor was then divided into two lots, one of which was boiled. Slices of lily tissue added to the respective solutions showed a marked swelling of the cell walls in the case of the unboiled extract, while in the boiled extract no change was observed.

Working along the line suggested by Ward (1888, p. 351) the writer made solutions of onion and prune juice. Five hundred cubic centimeters of the juice were placed in liter flasks which were then plugged, sterilized, and later inoculated with spores of the onion *Botrytis*. At intervals, ranging from four to twelve weeks, the mycelial crusts were removed with a sterile glass rod and thoroly ground in a sterile mortar with a very small quantity of washed, sterilized quartz sand. After grinding, enough sterile distilled water was added to give the mass fluidity when it was allowed to stand for an hour. The thick liquid, which was of a straw color and which had a sweet, sickening odor, was then filtered thru sterile filter paper into sterile glass beakers. Some of the beakers of liquid were placed over a gas flame and brought to a boil for one minute.

When the solutions were cool, discs of potato tuber tissue and thin slices of onion scale obtained aseptically were added. Checks were run by adding similar pieces of tissue to beakers containing sterile distilled water. At the end of three hours no change in the appearance of the pieces of tissue could be detected, but at the end of ten hours a very striking contrast between the tissue discs in the various solutions was plainly evident. The cell walls of the discs from the unboiled extract were greatly swollen in some places and laminated. They were not at all refractive. It was very difficult to detect the adjoining cell cross walls in some cases. The onion tissue cells did not show this change to as great an extent as did the potato tissue cells. No change could be detected in the tissue discs from the boiled extract, or from the distilled-water checks. These results agree with those obtained by Ward and De Bary. The experiments were discarded at the end of twelve hours because of possible bacterial action altho the presence of bacteria could not be detected microscopically at this time.

It was noted that when the fungal crusts were removed from flasks of liquid nutrient solutions an abundant growth did not reappear on the medium, altho numerous spores were present. Experiments with spores in hanging drops of the solution from the cultures showed that germination was retarded. This appears to be in accord with the observations of Lutz (1909) who found that *Botrytis cinerea* produced certain substances in nutritive solutions which retarded the germination and growth of that species of fungus. The products formed had many of the characteristics of enzymes.

Ward (1888, p. 348) tested the action of the liquid filtered from beneath the dense mycelial crusts of *Botrytis* growing on three different solutions and found that in hanging drops the unboiled filtrate had caused a distinct swelling of the cell walls of sections of lily bulbs. The writer made similar tests with the liquid secured from beneath dense mycelial crusts of the onion *Botrytis* growing upon a weak solution of onion and prune juices. Using a sterile pipette, quantities of the clear liquid were removed from the cultures at various ages and passed thru sterile filter paper into sterile beakers. Potato- and onion-tissue discs were then added to the boiled and unboiled filtrate and also to checks of sterile distilled water. The discs were removed and examined at the end of 6, 12, 24, 36, and 48 hours, but at the end of this time the writer was

satisfied that very little, if any, change could be detected in any of the solutions with the exception of the unboiled filtrate in which the tissue discs showed a certain amount of cell plasmolysis and possibly some change in the cell walls.

In this connection the results of the work of Reed (1912), Dox (1910) and Franceschelli (1915) are very valuable and interesting, since they also show that the mycelia of certain fungi possess active principles.

Action of droplets exuded from upper surface of cultures.—De Bary (1886) studied the pathogenic action of *Sclerotinia libertiana* and found that the active principle of the droplets of liquid exuded from the young sclerotia were thermolabile and therefore concluded that it was of the nature of a ferment.

Jones and others (1912) report the appearance of drops of a clear, brownish liquid exudate upon the surface of vigorous-growing cultures of *Phytophthora infestans*. These authors suggest that this is a normal bleeding process comparable to that observed by Pfeffer (1900). A quantity of the exudate was titrated to phenolphthalein and found to be slightly acid in reaction,—less so than the normal potato juice.

Ward (1888, p. 339) reports the extrusion of drops of liquid from the tips of hyphae at the margin of large mats of *Botrytis* mycelium grown on Pasteur's solution. He was able to obtain some definite reactions from these drops and concluded that they are made up quite largely of a soluble ferment which has the property of swelling and dissolving cellulose cell walls. The writer has observed drops of liquid, which may be very similar to those noted by Ward, extruded upon the upper surface of cultures of the onion *Botrytis* growing upon bread soaked with prune and onion juices. Similar drops were also observed upon onion-agar slants covered with a dense growth of the fungus. These drops varied in size from 1 to 3 mm. in diameter. They were of a clear, amber color, and rather thick and viscid. When tested with Millon's reagent negative results were secured. When placed upon blue litmus paper a strong acid reaction was indicated. In order to test the action of these drops upon the onion tissue some of them were taken up with a sterile 1 cc. pipette and placed upon the inner surface of onion bulb scales obtained aseptically and placed in sterile preservation jars, kept moist by the usual devices. At the end of three days the tissue of

the onion bulb scales upon which the drops were placed showed that dissolution of the middle lamella of the cells had taken place directly beneath the liquid. The cells were loosened and the wall wrinkled and folded. The contents of the cells were plasmolyzed and the tissue was discolored over an area of about 5 mm. around the edge of the drops. Sections thru these softened areas showed no fungus mycelium to be present. Checks were run by placing sterile distilled water on onion bulb scales similarly treated. These remained unchanged.

Action of extract from diseased bulbs.—De Bary (1886), Kean (1890), and more particularly Behrens (1898) studied the extract from diseased plant parts. All of these investigators demonstrated the activity of the extracts secured.

Voglino (1903) filtered thru a Pasteur-Chamberland filter the slimy mass obtained by squeezing garlic bulbs attacked by *Scle-rotium cepivorum*. The filtrate when placed on healthy bulbs caused destruction of the external tissues. This author finds this to be due to a "special substance" which infiltrates into the healthy tissue, thus separating the cells one from another.

To determine whether an extract from diseased onion bulbs had any effect upon healthy host tissue the following experiment was performed: A large number of onion bulbs attacked by the Botrytis were secured and the soft, diseased tissue cut off and placed in a mortar where it was ground to a pulp. Some care was exercised in cutting the tissue to discard all of that badly overrun by the fungus. The pulp was then transferred with the contained liquid to a bulb pipette which was sealed down to a Chamberland filter of the pencil type fitted to a suction flask. The liquid was then drawn down into the flask where it was permitted to digest for thirty-six hours. At the end of this time it was found to be sterile by plating out in media, and small quantities of about 1 cc. were withdrawn with sterile pipettes and placed upon onion scales. The onions from which the scales were secured were first cleaned by removing the outer scales, then immersing for two minutes in mercuric chlorid followed by sterile distilled water. The outermost scales were then peeled off and the inner ones secured as aseptically as possible with flamed forceps and scalpel and placed, concave side up, on crystallization dishes in large, sterile preservation dishes. Checks were run by placing sterile distilled water on some of these

onion bulb scales. At the end of forty-eight hours, when the greatest change was evident, the scales to which the extract had been added showed the tissue beneath the liquid to have a dark, water-soaked appearance thru the entire thickness of the scale. Fig. 2, Plate X, shows a photograph of one of these scales, the under or convex side of the scale being shown here. Sections thru the spots over which the liquid had remained showed the cells to be loosened and plasmolyzed, and, in some cases, the walls were much laminated and folded. When a piece of this tissue was placed on a slide and covered with a cover glass, gentle pressure separated the cells very readily. The cheeks remained unchanged. No evidences of bacterial action could be detected with the microscope. It was plainly evident that a substance obtained from the diseased onions passed thru the filter used, and had a marked influence upon the host tissue.

In another series of experiments about 5 cc. portions of the extract, which had passed thru the filter, were placed in sterile Erlenmeyer flasks. To these were added discs from onion scales obtained aseptically. The discs were 1 cm. in diameter and the thickness of the bulb scale. Checks were run by using sterile distilled water in place of the extract on another lot of discs. In twenty-four hours the discs in the extract showed marked loss of coherence, that is to say, when the discs were pulled or pinched between the fingers the tissue appeared to be softened. Microscopic examination confirmed the macroscopic observations in that it showed the cells to be loosened along the line of the middle lamella.

Using the filtered extract noted above, about 1 cc. was injected into each of three healthy onion bulbs. Five days later when the bulbs were cut open the results appeared to be negative with the exception of one bulb which showed a marked discoloration of the tissue along the track of the needle.

The results of these experiments appear to give strong evidence of the production of an enzym or enzymes by the fungus.

Action of the extract from young mycelium.—In spite of the fact that the foregoing results point to the constant production of an enzym the active principle displayed by the products prepared from the old mycelium were not as active as was believed to be the case with the young, advancing hyphae of the fungus. The logical procedure, then, appeared to be the preparation, if possible, of an

extract of the young elements of the fungus, since Dox (1910) stated that the time to prepare the intracellular enzymes is before fructification.

Brown (1915), in his painstaking study of the physiology of parasitism of *Botrytis cinerea*, immersed thin discs of tissue from various plants in a strong extract made from the germ tubes of that fungus. In his work he was able to demonstrate that the extract, which he inactivated by heating, by mechanical shaking, and by neutralization with alkali, possessed a two-fold action on the plant tissue. It produced a macerating action by dissolving the middle lamella, and produced death of the cell by its action on the protoplast. These two actions are thought by this author to be possessed by the enzym found in the extract.

Stevens (1916) in making a careful study of the pathological histology of strawberries affected by a species of *Botrytis* accepts Brown's conclusions as to the conditions found in the cells attacked by the strawberry fungus.

The method as employed by Brown was found to be applicable to the problem at hand and was followed, with some modifications. In short, the method used by the writer was as follows: An abundant supply of spores was secured by growing the fungus upon onion agar¹ which was found to be the most satisfactory of the various kinds of media tried.

Botrytis spores for inoculation were secured from cultures made from isolations from diseased onions from storage houses in New York. Previously inoculated tubes of onion agar were poured into the ordinary petri dishes. A copious spore production took place upon such plates and in eight to ten days the spores could be readily removed by covering the plates with sterile distilled water, and then by the use of a sterile glass rod bent at a slight angle at one end, the spores together with a slight amount of the aerial portions of the cultures, were brushed off. This material was then filtered thru a piece of sterile linen having 19 threads per linear centimeter, into a sterile centrifuge tube. The spores readily pass thru with the water leaving the fungal debris upon the filter. After centrifuging for two minutes at a speed of 2000 revolutions per minute the spores were thrown to the bottom of the tube where the wet volume was noted. The water was carefully siphoned off and the

¹ See footnote page 401.

centrifuged spores were suspended in a nutrient solution made by autoclaving cleaned onions for fifteen minutes and then using the sterile juice. The spores were suspended in this onion juice at the rate of 0.5 cc. wet volume to 50 cc. of the nutrient liquid. This spore suspension was then sown on circular glass plates eight inches in diameter, supported on three large corks in large, sterile, deep, culture dishes. A small amount of water was placed in the bottom of the culture dishes. After being carefully leveled and sterilized the glass plates were ready for seeding. This was done by distributing 5 cc. of the spore suspension very evenly over the plates by the use of a sterile, fine pointed, 5 cc. pipette and a sterile glass rod bent at a slight angle at one end. After thirty-six hours at a temperature of 22°C ., a copious spore germination had taken place producing a continuous web of germ tubes. This web was readily peeled or scraped off with a sterile microscope slide and thrown upon a sterile filter made of a linen handkerchief placed over a ring stand. After being vigorously washed under the water tap for one-half hour to remove all of the culture medium, ungerminated spores, etc., it was treated to four washings with sterile distilled water, squeezed as dry as possible, then spread out upon sterile pieces of filter paper which were placed upon squares of the kind of netting known as hardware cloth and lowered into desiccators containing calcium chlorid. In from twenty-four to thirty hours this fungus material was dry. It was then scraped off, carefully weighed, and mixed with an equal weight of sterile, washed, clean, dry sand. This material was then ground to a fine, light gray powder. This powder had a distinct odor of fresh mushrooms. In order to obtain an active extract, this spore material or powder was extracted in the proportion of 0.2 grams of the powder to 3 cc. of sterile distilled water. After one hour's extraction the liquid was cleared by centrifuging for three minutes at 3000 revolutions per minute, decanted, and again centrifuged and decanted. This gave a liquid having a light straw color and an odor resembling mushrooms.

Tests were then made with this crude fungal extract as to its ability to destroy the coherence of onion and other plant tissues placed in it for various lengths of time. Brown (1915), in his investigations, tested the degree of coherence by pulling the discs of tissue with the hands. In order to eliminate the personal equation

which occurs when the coherence is tested in this manner and to obtain some definite numerical values for strength of tissue affected by the extract at stated intervals of time, a chemical balance such as is used for rough weighings was utilized. The pan was removed from one side of the balance and a contrivance consisting essentially of two clamp clothes pins, the jaws of which were covered with corrugated rubber, was substituted. After being counterpoised, the tensile strength of pieces of onion and potato tuber tissue of uniform thickness and width could be determined with a surprising degree of accuracy in this crude piece of apparatus by pouring sand into a receptacle on the opposite pan, the sand being weighed after each test. Pieces of tissue for the purpose of these tests were very easily prepared by cutting strips of onion leaves 7 mm. in width, also by cutting the same width strips of the thin membrane found between the scales of the onion. Strips of potato-tuber tissue of uniform thickness (0.5 mm.) and width (7 mm.) were also used. Two safety-razor blades securely clamped to a piece of hard wood 7 mm. in thickness served very nicely to cut the strips of uniform width. A sliding microtome was used to cut the desired thickness of tissue. The pieces of tissue were freed from contained air by injection, under the air pump, with water. They were then placed in the extract and removed at intervals and tested for coherence. Checks were provided in all cases by using sterile distilled water in place of the extract on part of the tissue discs.

Effect on tissue strips. — After a considerable number of preliminary experiments, all of which gave uniformly satisfactory results, the following experiment was performed. A detailed record of this experiment will suffice to make clear the methods used and aid in interpreting the results secured. Values of tests for coherence are recorded in round numbers in grams, i. e., in terms of grams of sand required to break a certain width and thickness of tissue after being acted upon by the fungal extract for a given length of time. Where time permitted of the securing of more than one value for a tissue the average of the value is given.

A series of tissue discs in quantities of the boiled extract was carried along with the above series. These discs showed approximately the same values at the end of the twelve hours as at the beginning. They were practically the same as the checks. All of the tests were discontinued at the end of the twelve hours since loss of

TABLE IV.—EFFECT OF THE FUNGAL EXTRACT UPON ONION-LEAF AND SCALE-MEMBRANE TISSUE, AND UPON POTATO-TUBER TISSUE.

TIME.	PLACED IN STERILE DISTILLED WATER.			PLACED IN THE FUNGAL EXTRACT.		
	Onion- scale. mem- brane.	Onion- leaf tissue.	Potato- tuber discs.	Onion- scale mem- brane.	Onion- leaf tissue.	Potato discs.
	<i>Gms.</i> ¹	<i>Gms.</i> ¹	<i>Gms.</i> ¹	<i>Gms.</i> ¹	<i>Gms.</i> ¹	<i>Gms.</i> ¹
Initial reading, average....	78	365	122	77	363	127
30 minutes.....	79	370	115	79	360	113
45 ".....	71			83		
1 hour.....	76	345	130	80	355	101
1 " 15 minutes.....	80			67		100
1 " 45 ".....	69	340	123	57	340	95
4 hours.....	76	350	125	34	301	33
6 ".....	76	355	134	30	210	27
12 ".....	70	338	120	² Soft	200	² Soft

¹ Weight required to destroy coherence of strips 7 mm. wide of onion leaves, of membrane separating onion bulb-scales and of potato-tuber tissue 0.5 mm. thick.

² By "soft" is meant that the tissues had so lost coherence that they could not be handled or placed on the balance to be tested as to their strength.

coherence had occurred in some of the tissues before the expiration of this time. The possibility of bacterial action appears to have been eliminated in this period of time, since no bacteria were revealed by the aid of the microscope.

The writer is satisfied that complete loss of coherence in many instances took place in less time than 12 hours; however, a weighing of approximately 20 grams at six hours was the least that could be satisfactorily made with these tissues which were rapidly becoming too fragile for this purpose.

From the above table it is seen that the strips of onion-scale membrane showed a decrease in numerical value from 77 to 30 in 6 hours and a complete loss of coherence in 12 hours. The onion-leaf tissue showed a decrease in numerical value from 363 to 210 in 6 hours and to 200 in 12 hours. The potato-tuber discs showed a decrease in numerical value from 127 to 27 in 6 hours and a complete loss in 12 hours. The checks apparently remained unchanged. These data are shown graphically in Fig. 1 (p. 432). The curves appear typical for enzymes. In addition to data secured on the strips of green-onion-leaf tissue it was noted that a marked loss of color took

place in from 30 to 60 minutes after exposure to the action of the extract. This change in color appeared to be due to the action of the extract on the protoplast. It should be noted that because of the presence of the epidermis the extract could attack these pieces of tissue readily on but one side.

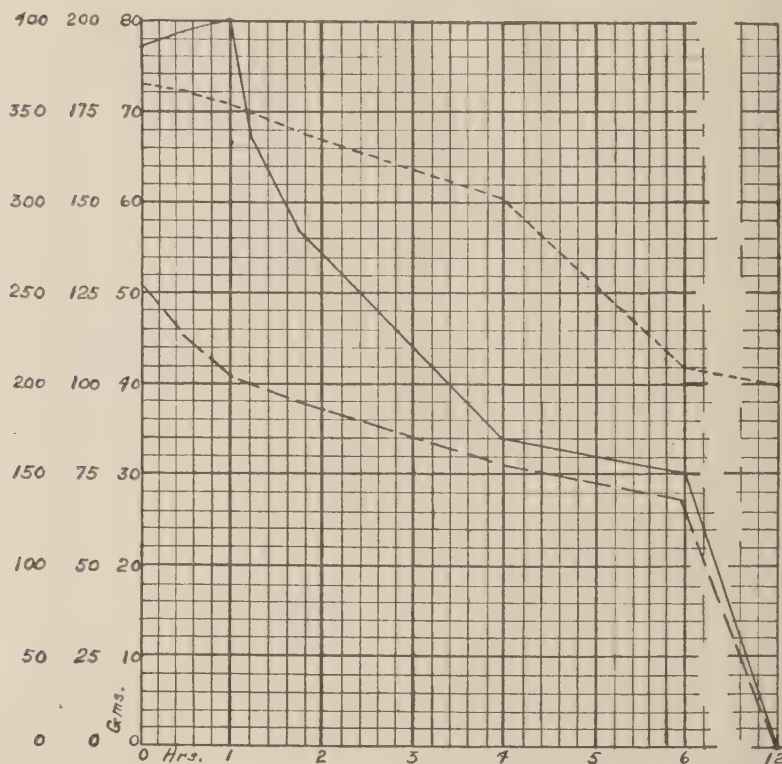


FIG. I.—CURVES SHOWING ACTION OF ENZYM (FUNGAL EXTRACT) ON PLANT TISSUES, (————) ONION-SCALE MEMBRANE, (-----) ONION-LEAF TISSUE, (- - - -) POTATO-TUBER DISCS.

The data from these tests, together with the evidence secured by examinations of the tissue with the microscope, seem to yield but one conclusion, namely: That the action of the extract is of a two-fold nature, — (1) solution of the middle lamella resulting in loss of coherence followed by (2) disintegration of the remainder of the

cell. Solution of the middle lamella allows the tissue to fall apart readily and the subsequent disintegration of the cell changes the tissues to a structureless mush. The final stage, however, does not appear to be brought about as readily as is the preceding one. The action of the extract upon the tissue was in all cases quite similar to that induced by the action of the fungus itself upon the host tissue.

Injection of the extract.—One cc. portions of the standard fungal extract were drawn up into a sterile hypodermic syringe and then injected into firm, healthy onion bulbs, using all precautions to secure asepsis. When the bulbs were opened five days later it was found that the tissue along the scales, for some millimeters, showed a brownish, water-soaked condition. Examination of the discolored tissue under the microscope, and comparison with apparently unchanged tissue from the same bulb, showed the cells to be loosened along the line of the middle lamella and badly plasmolyzed. The cells came apart very easily.

Action of the extract upon cotton fibers:—Five cc. portions of the fungal extract were placed in sterile test tubes. Cotton fibers or threads, also small strips of Schleicher and Schull's filter paper were then added to the extract. Observations were made from time to time over a period of forty-eight hours when the experiment was discarded. Altho there appeared to be a weakening of the tissue the results are too indefinite to permit any conclusions to be drawn.

A study of some of the physical and chemical properties of the extract.
Effect of temperature upon the activity of the extract.—Quantities of the fungal extract were heated to the boiling point, then cooled and their action upon onion and potato tissue compared with that of the unboiled extract. It was found, as previously noted, that boiling completely destroys both the macerating and killing action of the extract. The action of the extract upon the tissues was practically inhibited by heating to 65° C.

Effect of age upon the action of the extract.—The dry combined sand and fungal elements composing the fungal powder were permitted to stand for over two months in a glass bottle closed with a ground-glass stopper. At the end of this time the powder was extracted in water and its action upon the plant tissues used was equally as effective as the fresh fungal extract. This is to be expected in view of the statements of Michaelis (1910). Jones (1909, p. 327)

and Spieckermann (1902, p. 166), studying the enzymes produced by phyto-pathogenic bacteria, report that they were unable to note any loss in enzymic activity in dried, enzyme-containing precipitate kept for months or even years. Dox (1910, p. 391) stated that the enzyme powder, if stored in a glass-stoppered bottle and kept from the light will retain its activity indefinitely.

Titration of the extract.—When five cc. portions of the standard fungal extract were added to 45 cc. of distilled water and titrated with N/20 NaOH, using phenolphthalein as an indicator, it required an average of 1.1 cc. of the N/20 alkali to neutralize the acid in the 5 cc. Therefore the extract was +11° acid in terms of Fuller's scale or 1 per ct. normal acid to phenolphthalein.

Relation of chemical reaction to activity.—Brown (1915, p. 338–341), in his work with fungal extract of *Botrytis cinerea*, has shown that when the acidity of the extract was diminished by adding N/1 NaOH “the activity remained unaltered up to a certain point, when it falls sharply to zero.” This author also found that in all cases when the acidity of the extract was increased by the addition of an organic or a mineral acid up to a certain point, the action on the activity of the fungal extract was one of retardation. In an alkaline solution the extract had no activity.

Jones (1909, p. 329) found that 2 per ct. alkali slightly inhibited, while 10 per ct. totally inhibited the action of the enzyme pectinase of *Bacillus carotovorus*. In the case of acids a reaction of 1 per ct. normal acid and above inhibited the action, while 5 per ct. to 10 per ct. totally inhibited the action of this enzyme.

Experiments bearing on this point were carried out by the writer. They are interesting and significant. The results of a single experiment will suffice to illustrate the method and the results. A quantity of the fungal extract was prepared by the usual methods as given above. Ten cc. portions of the extract were placed in each of four sterile beakers. One lot was left untreated. One lot was neutralized by adding 0.1 cc. of N/1 NaOH since it was found that with this particular lot of extract each 5 cc. portion in 45 cc. of distilled water required 1.1 cc. of N/20 NaOH to neutralize the acid, using phenolphthalein as an indicator. One lot was made alkaline by adding 0.1 cc. of N/1 NaOH to neutralize it, then adding an additional 0.1 cc. making the extract –11° Fuller's scale. When the alkali was added the color of the extract changed from the

characteristic pale straw color to a clearer yellow with a faint tint of amber. One lot was made acid by adding two drops (about 0.1 cc.) of N/1 HCl thereby making the extract about $+20^{\circ}$ acid Fuller's scale. Checks were run by using distilled water in place of the extract. Strips of the thin membrane from between the onion scales and potato tuber discs cut with a microtome were then added to each of the lots of extract and the checks. The following table shows the results secured:

TABLE V.—RELATION OF REACTION OF EXTRACT TO ITS ACTIVITY.

REACTION OF EXTRACT.	TIME IN HOURS.					
	1 hour.	2 hours.	3 hours.	4 hours.	5 hours.	6 hours.
Untreated.....	Un- changed.	Slightly softened.	Slightly softened.	Becoming very soft.	Coherence almost lost.	Total loss of coherence.
Neutral.....	Un- changed.	Slightly softened.	Slightly softened.	Slightly softer.	Softened con- siderably.	Almost as soft as the untreated lot.
Acid.....	Un- changed.	Un- changed.	Very slightly softened.	Slightly softened.	Slightly softened.	Slightly softened.
Alkaline.....	Un- changed.	Un- changed.	Un- changed.	Un- changed.	Un- changed.	Un- changed.
Checks.						

The results of this and similar experiments agree quite closely with those secured by Brown. Plainly, there appeared to be but little difference between the action of the neutralized and the untreated extract. No change took place in the tissues in the alkaline solution. At certain points of acidity the action of the acid extract was retarded quite markedly. The action of the extract upon the onion tissue, in all cases where it could be detected, was quite similar to the action of the fungus itself upon the onion bulbs.

An attempt to secure and purify the enzyme by precipitation with alcohol.—All of the preceding experiments seem to give conclusive evidence that an enzyme is produced by the onion *Botrytis* which has a pronounced effect upon the tissue of the host. The following studies were made in an attempt to secure the enzyme by precipitation with alcohol.

Methods.— A quantity of the fungal extract was prepared by the methods previously given. This was carefully measured and enough 95 per ct. alcohol added to make the solution 80 per ct. alcoholic, since Jones (1909) has shown that with the enzym pectinase the best results were secured with this concentration. Immediately after the alcohol was added, a medium to heavy, white, flocculent precipitate was formed thruout the liquid. In the course of a few hours, this precipitate had gradually settled to the bottom of the graduate. The clear liquid was then siphoned off, and the remainder poured upon a sheet of high grade filter paper. The liquid passed thru readily leaving the precipitate on the filter paper where it was washed with absolute alcohol. The filter paper containing the precipitate was then held for a few minutes in a current of dry, warm air from an electric oven. When partially dry the paper and precipitate were lowered into a desiccator over sulphuric acid. After remaining in the desiccator over night, the gray precipitate was scraped from the paper and powdered. The powder was then redissolved in sterile distilled water, using 50 cc. to about 0.2 gram of the precipitate. After standing for four hours with occasional shaking the powdered precipitate appeared to be entirely dissolved. Discs of onion-bulb and potato-tuber tissue were then added to the solution and it was found that in the course of four hours softening of the tissues had taken place. The softening continued until the pieces of tissue lost their coherence. When viewed under the microscope the cells were separated, their contents plasmolyzed and granular. The cell walls were wrinkled and folded. This experiment was repeated with the same results.

Effect of temperature upon the dissolved precipitate.— Tests were made of the action of boiled and unboiled dissolved precipitate upon tissues. The boiled solution showed no noticeable action upon the tissue discs, while the unboiled solution showed pathological changes as noted in the preceding paragraph.

Reprecipitation of the enzym.— After using the dissolved precipitate solution for some of the tests noted above, the tissue discs were removed and the solution made 80 per ct. alcoholic when a white, flocculent precipitate was secured quite similar to that observed when the fresh fungal extract was made alcoholic. To further test the action of the reprecipitated enzym, a sufficient volume of the fungal extract was secured. This was made 80 per ct. alcoholic

and the usual precipitate secured in due time. The precipitate was dried, powdered, and redissolved in water. This process was repeated three times to the point where the powdered precipitate was redissolved in water, when tissue discs were added. The action of this triple-reprecipitated and dissolved precipitate upon the tissues exposed was apparently the same as the former solution tested except that its action was somewhat retarded. In other words, it retained its active principle thruout this treatment, without appreciable loss of cytolytic action thru precipitation or re-solution. No diminution in the amount of precipitate was apparent.

Precipitation of the enzyme from old mycelium.— The mycelial crusts, or wefts of fungous growth upon flasks of prune juice, were lifted off with a sterile rod and forceps at the end of 45 days and placed in a sterile mortar. A small quantity of sterile, clean sand was sprinkled over the mass, which was then ground to a pulp. Enough sterile distilled water was added to give it fluidity when it was poured upon a filter consisting of high-quality absorbent cotton placed over sterile cheesecloth. This was then squeezed. The liquid which passed thru was of a straw color and had a sweet, sickening odor. Fifteen cc. portions of this were then placed in graduates and 80 cc. of 95 per ct. alcohol added to each. Just as soon as the alcohol was added a thick, stringy, rubbery precipitate, having a dirty white color was formed. If left undisturbed this precipitate, which was thought to be of a proteinaceous nature, settled to the bottom of the graduate and took on a brownish white color. Using a glass rod this heavy precipitate was very easily collected and removed from the solution. In either case, whether removed or permitted to remain, a second white precipitate appeared in the course of approximately one-half hour's time. In the case of those lots from which the first heavy precipitate had been removed, this white, flocculent precipitate was readily filtered off by means of filter paper. This second precipitate was dried, powdered, and redissolved in water exactly as was done with the precipitate from the fresh fungal extract as given on a preceding page. When redissolved in sterile distilled water its action upon onion and potato tissue appeared to be similar in all respects to that of the precipitate from the fresh fungal extract. This precipitate was readily redissolved, reprecipitated and redissolved. Ward (1888, p. 353) appears to have secured quite similar results with his lily *Botrytis*. In this

connection it should be noted that when a small piece of the fungal crust was lifted from the prune-juice-culture flask and floated upon the surface of an 80 per ct. alcoholic solution a fine, white precipitate was noticed in the region of the lower surface of the crust or weft. The writer is not prepared to state whether this precipitate was due to the presence of the enzym or was precipitated proteinaceous matter.

Conclusions.—The results obtained from a study of the action and properties of an extract from the old and young *Botrytis mycelium*, and a consideration of the possibility of oxalic acid being the toxic substance excreted, forces one to conclude: (1) That the fungus secretes a middle-lamella-dissolving enzym. At least, the active principle of the extract from the old and the young triturated mycelium is to be found in the alcoholic precipitate and responds to many of the physical tests used for the detection of enzymes. (That both the macerating and lethal actions upon the host are due to the same enzym has not been proven since the precipitate secured from the fungal extract may have consisted of two or more enzymes.) (2) That the toxicity of the extract is not due to either oxalic acid or oxalates, since tissue discs in oxalic acid solutions show much less marked effect than similar discs in the fungal extract, when length of time and pathological changes in tissue are considered.

If we accept the work of Cross and Bevan (1895), Green (1901), Allen (1901), Bigelow and others (1905) as to the composition and origin of the middle lamella, and the results of Fremy (1840, 1848) and Bourquelot and Herissey (1898) as to the enzym capable of hydrolyzing the pectic elements in the middle lamella, we must adopt the nomenclature of Jones (1909), Euler (1912) and the classification given by Haas and Hill (1913) and refer to the middle-lamella-dissolving enzym produced by this fungus as pectinase.

Action of the fungus on cellulose. — From a study of the diseased or fungus-invaded tissue of the onion bulbs it seemed evident that the cellulose walls were attacked by the fungus or its enzymes. An attempt was made to determine whether the fungus produced an enzym capable of attacking cellulose by employing the following methods: The fungus was grown upon solutions poor in organic matter with and without the addition of cellulose. The solution which gave the most satisfactory results was that employed by Reed (1912) and was made up as follows:

Ammonium nitrate.....	10.0 grams
Dipotassium phosphate.....	5.0 "
Magnesium sulphate.....	1.0 "
Sodium citrate.....	1.0 "
Distilled water.....	1000 cc.

Working along the line suggested by Coons (1916) filter-paper cones were placed in small culture dishes. Ten cc. portions of the solution were then added to each of the dishes containing the filter paper, and also to an equal number containing no filter paper. The two lots were then autoclaved. Inoculations were made with spores, a few dishes of each lot being left uninoculated as checks. In four weeks' time a copious growth showing abundant conidium formation, had taken place on the filter paper cones, but only a very small web of the mycelium developed in the dishes without filter paper.

In addition to the cultures in small culture dishes, 500 cc. Erlenmeyer flasks were used. Two hundred cc. of the solution were placed in each. To one of each pair of flasks 2 grams of Schleicher and Schull's filter paper were added, both were then sterilized in the autoclave and inoculated. There was considerable mycelial growth and some spore production on the filter paper, but only a minute tuft of mycelium on the solution without the filter paper.

After 90 days' growth the solutions beneath the cultures were titrated with N 20 NaOH using phenolphthalein as an indicator. The solution to which the filter paper had been added and upon which the copious growth occurred showed a titer of $+47^{\circ}$ acid Fuller's scale. The titer of the solution to which no filter paper was added was $+31^{\circ}$ Fuller's scale. Neither the culture flasks with the filter paper nor the controls without paper showed any reducing action upon Fehling's solution, indicating that the small amounts of sugar, if formed, were probably used immediately by the fungus.

These results with the filter paper cellulose appear to be strong evidence that the fungus produces an enzym, cytase, which has the power of attacking cellulose. This may in part explain the action upon the cell walls of the host tissues as noted elsewhere. In this connection it is worthy of note that species of *Botrytis* have been reported as attacking cellulose by De Bary (1886), Ward (1888), Miyoshi (1894), Behrens (1898), Van Iterson (1904), and Ruhland (1908). Experiments dealing with the demonstration of cytase

production by the fungus are not completed. These will be reported at a later date.

IMMUNITY RELATIONS.

Relative resistance of different varieties of onions.—It is a fact well known to onion growers that the thin-skinned white varieties suffer very severely from the neck rot. There appears to be but little difference between the relative resistance of the red and yellow globe varieties to the neck rot. However, it is believed by some growers that the red varieties are not as easily affected. The difference in these cases appears to be partly one of degree of maturity and not a varietal difference. No difference in susceptibility to neck rot could be detected between the various shapes of onion bulbs whether round, flat or globe shaped; however, it was found that the extreme globe or "bottle neck" types were slower in drying out at the neck and consequently showed a greater percentage of neck rot.

CONTROL.

FACTORS WHICH FAVOR INFECTION OR AMOUNT OF NECK ROT.

MATURITY AND DEGREE OF CURING.

The condition of the bulbs when ready to harvest has an important bearing upon the amount of neck rot which may develop in storage. Bulbs which have large green tops, and "bull-necks" are especially susceptible to neck infection. It is preferred that the tops ripen down and shrivel, and the outer skin be dry before pulling the crop. In this connection the amount of onion mildew (*Peronospora Schleideniana*) has an important bearing upon the amount of neck rot which may develop in storage. Onions attacked by the mildew fungus are as a consequence poorly matured and soft and are especially susceptible to the neck-rot disease. This fact was strongly emphasized by one prominent grower who said that if they could get their onions thru the season without being attacked by the mildew, they would not lose so heavily from the rot in storage.

FERTILIZERS.

Fertilizers, so essential in the production of a crop of onions, were found to have no noticeable relation to the amount of neck rot except in instances where applied in incorrect proportions or very

late sowings were made, or where large quantities of manure have been applied to the land just previous to planting. These practices result in an over-production of tops which die late in the season affording an excellent opportunity for infection thru the dying necks. The bulbs from such plants are usually small and soft, and later show a high percentage of rot in storage.

SOIL TYPES.

Early in the study of this disease it was believed that the fungus was more prevalent in the muck soils, since muck onions showed a comparatively larger amount of rot. It was found, however, that the amount of neck rot depended largely upon other factors, mainly degree of maturity of bulbs, extent of curing and the weather at curing time, and the storage conditions under which the onions were placed. Upland onions often die and dry down earlier in the season, and the bulbs are often firmer as a result. A number of instances have been noted where red onions growing upon upland of a loamy character have shown considerable neck rot in storage. One instance came to the writer's attention where the sclerotia of the fungus were found upon the bulbs grown upon a loamy soil, at the time of placing them in the storage house in early fall. None of the onion growing regions visited by the writer were found to be entirely free from the neck-rot disease.

AIR DRAINAGE.

Air drainage of the onion fields appears to be an important factor. One small onion-growing section was studied in particular since it had the reputation of consistently yielding onions which later developed considerable neck rot in storage. The factors at work here were plainly the high hills and dense woodland on two sides of the onion fields. The dews and rains did not evaporate readily and the crop remained wet during the greater part of the forenoon. This is especially detrimental during the harvesting and curing periods.

STORAGE CONDITIONS.

Careful investigations have shown that of all the various factors considered which have a bearing upon the development of the neck-rot disease, the control of storage conditions is the most important.

The factors which favor onion neck rot are overheating and poor ventilation in storage houses. It must be admitted, however, that the very best houses cannot carry a crop safely thru the storage season when the bulbs are soft and poorly cured, and moist weather prevails soon after storage begins. A considerable number of the houses used for onion storage are entirely unsuited for this purpose. One particular storage house, having a capacity of approximately 20,000 crates and in which several thousand crates were lost thru neck rot in 1915, was visited by the writer for the purpose of securing some data as to the relative humidity of the house. This house was of concrete construction. It had a rather low, flat roof, only three or four doors along each side of the building and very meager provisions for ventilation thru the roof. The house has single walls and is heated by steam, the pipes running along the outside walls. As cold weather came on and steam was passed thru the heating system it vaporized all the frost on the walls and pipes, the moisture passing thruout the stored onions bringing on a considerable amount of neck rot in the course of a few days.

Ventilation is of prime importance. The roof should be of a steep pitch, and preferably double, to draw out the moist air. The proper regulation of the ventilation vents is important. These should be closed during damp, foggy, misty weather. The writer traced a severe epidemic of neck rot in one storage house to the fact that the owner opened the doors and left them open during a warm, misty day in mid-winter while a carload of onions was being sacked and removed from the storage house.

Records of the temperature and humidity in onion storage houses were obtained by the use of carefully adjusted Lambrecht polymeters. These records show that a relative humidity of 65-70 per ct. coupled with a temperature of 16-22° C. is very detrimental to onions in storage.

A study of the temperature relations of the fungus show that the temperature of the storage house must be kept very close to 0° C., or about 34° F., in order to prevent the development of the neck-rot fungus. Ames (1915) has shown that a temperature of near zero Centigrade (32° F.) must be maintained to prevent the development of rot fungi in refrigerating experiments.

The onion storage house at South Lima, New York, operated by Mr. C. C. Mowris, was placed at the writer's disposal for the purpose of storage observations. Very little neck rot occurred here. This house, which is one of the best, shows a very desirable type of construction providing ample means for ventilation and heating (Plate XI, fig. 2).

STORAGE EXPERIMENTS.

Occasionally one finds notices posted on onion warehouses to the effect that "no onions having necks or stems longer than one and one-half or two inches will be accepted." Because of the fact that some onion buyers lay particular stress on the length of neck of onions for storage, claiming that long-necked onions do not keep as well as the short-necked ones, some tests were made upon this point. On September 24, 1913, the writer visited a field in which some neck rot had occurred the previous season, and also during the present season, in crates of onions then in windrows. Six crates of these onions were prepared for an experiment. In two the bulbs were topped very short; in two they had necks 3-4 inches long; while in the remaining two the tops were left uncut. Three of the crates (one of each pair) were stored in a very dry, cool place (the loft of a large building). The other three were stored in the rather damp basement of a storage house where the ventilation was very inadequate tho similar to that of some onion storage houses. These two lots of onions were kept in these storage places until March 20, 1914, and data secured from time to time as to the number of bulbs affected with neck rot. A summary of the data shows that an average of approximately 45 per ct. of the bulbs in the damp storage were destroyed by the neck-rot fungus and the remainder sprouted. Of the bulbs in the very dry storage between 2 and 3 per ct. were destroyed by the neck-rot fungus while only a few bulbs sprouted. As to the effect of short versus long topping and tops uncut upon the amount of neck rot no data could be secured since all were rotted alike in the damp storage. In these experiments the length of neck did not appear to be an important factor, the humidity of the storage place being the factor which influenced keeping quality.

A similar experiment with mildewed onions was carried along with the above-mentioned series and as with the normal crop onions

the length of neck proved unimportant; the humidity of the storage quarters determined the keeping quality. The mildewed onions showed neck rot quicker in the damp storage than did the normal crop. It was evident that the mildewed crop was not as mature and carried more moisture into the storage with it.

The results of these experiments indicate that the length of neck, whether one or three inches long, has no relation to the amount of neck rot.

In an attempt to determine how soon after being placed in storage the neck rot would appear upon the onion bulbs, an experiment was conducted in which thoroly ripe and immature bulbs were compared. On September 3, 1913, crates of ripe and of immature onions were taken from windrows in an onion field in Livingston County, New York, and immediately transported to an onion storage-house in which considerable neck rot had occurred the previous season. On September 24, or 21 days later, the neck-rot fungus could be found fruiting on a considerable number (about 10 per ct.) of the bulbs in this rather damp storage house even tho the bulbs were in new crates not used for onions before. The immature bulbs showed a greater development of the disease.

These and similar storage experiments emphasize two important points: namely, that the crop must be dry and mature and stored in a dry, well ventilated house in order to materially check the development of the neck-rot fungus.

STUDY OF REMEDIAL MEASURES.

SPRAYING.

As to the effect of spraying onions with bordeaux mixture upon the amount of neck rot which developed in storage the writer has the following data to offer. Thru the courtesy and cooperation of Mr. Henry Greffrath of South Lima, N. Y., the crops from two fields of onions, one sprayed with bordeaux mixture, the other unsprayed, were placed in storage and when the stock was sold at the end of the storage period observations were made on the amount of shrinkage, due mainly to neck rot and sprouting. The data are as follows: 2315 bushels unsprayed onions sold March 1, 1914, gave 15.2 per ct. shrinkage; 942 bushels sprayed onions sold April 8, 1914, gave 10.8 per ct. shrinkage, a gain of 4.4 per ct. due to spraying three times with 5-5-50 bordeaux mixture. It is to be noted, however, that the

sprayed onions were removed from the storage over one month later than the unsprayed crop. It is also worthy of note that the sprayed stock was topped by a topping machine while most of the unsprayed stock was hand topped. Attention has already been called to the fact that many onion growers claim that hand-topped onions will keep better than machine-topped stock. It may be well to state that both lots of onions were stored in the same storage house and under the same conditions and that the storage was slightly overloaded which is very unfavorable to long keeping.

The spraying outfit used by Mr. Greffrath was one designed by himself for the purpose of spraying celery and onions. It consisted of a boom carrying fourteen nozzles eighteen inches apart, attached to a field power sprayer by two leads of hose. The sprayer is driven along the alleys, which usually consist of two rows of celery or lettuce between wide strips of onions, and the boom is carried by two men over the onion rows. No resin sticker was used in the bordeaux mixture since it was found that by going over the rows twice, i. e., once each way after short intervals of time, carrying the boom with the nozzles pointed slightly forward, and using high pressure (200 lbs.), the onion foliage could be completely covered by the fine, mist-like spray.

In a series of smaller experiments conducted at the Geneva Station, in which no data as to yield were secured, it was found that for a combination spray for onion mildew, neck rot and thrips, 5-5-50 bordeaux mixture to which were added Black Leaf 40 at the rate of three-fourths pint to 100 gallons of the spray mixture, and soap at the rate of 5 pounds per 100 gallons could be used with comparative safety on onions. A very slight discoloration of the leaves occurred where the mixture collected in drops. The nicotine extract in the mixture appeared to be quite effective against the thrips.

There are several serious difficulties met with in the spraying of onions which tend to make onion spraying very unpopular among onion growers. The most important of these difficulties is the tendency of the tops of the plants to drop over and become entangled just at the period when fungus diseases appear, making it very difficult to pass thru the field. Also on account of the glaucous character of the onion leaves, it is very difficult to make the spray stick to the foliage when using one of the low-pressure outfits even when the resin sticker is added.

Clinton (1904) reported some spraying experiments designed to determine the value of bordeaux mixture as a preventive of neck rot on white onions. Three applications of the spray mixture were made, but since no neck rot developed in storage no data were obtained upon the relative keeping qualities of the sprayed and unsprayed stock.

FUMIGATION.

The fumigation of onions with formaldehyde gas, either just before or just after they are placed in storage houses, has been recommended by Selby (1910) and Humbert (1916) as an efficient method of preventing the neck-rot disease. In order to test the efficiency of the gas against the neck-rot disease, quantities of onions, varying from a few specimens to one bushel lots were subjected to the treatment for twenty-four hours. The fumigation chamber used was a wooden box of 21.56 cubic feet capacity constructed of matched lumber and painted on the inside. The fittings of the door, which was on one side, were perfectly tight and but very little odor of escaping gas could be detected during the experiments. The gas was generated by the permanganate method, using 30 cc. of formaldehyde solution and 14.25 grams of potassium permanganate for the 21.56 cubic feet of space. These quantities are equivalent to 23 ounces of permanganate and 3 pints of formaldehyde per 1000 cubic feet of space as recommended by Morse (1907) for the disinfection of potatoes. The details of all of the seven tests with gas will not be given. The results of one or two typical tests will suffice to illustrate the results secured. On October 2, 1913, a Georgia peach basket, containing about forty onions of both red and yellow globe varieties secured directly from the field where they were cured and made ready for the storage, was placed in the fumigation chamber. The charge of chemicals was prepared, mixed and the door closed immediately. The charge of gas was permitted to remain in the chamber for twenty-four hours when the door was opened and the basket of onions removed. Upon examination it was found that the gas had injured the onions where the outer dry skins had peeled off exposing the flesh of the scales. The injury was in the form of sunken areas or pits (Plate VIII, fig. 3) and in some cases appeared as distinct blisters. During the experiment the fumigation chamber stood in a storage barn where the conditions were practically identical with those found

in an onion storage house. After an examination was made the basket of onions was immediately taken to a potato storage cellar where it remained thirty-nine days. At the end of this period it was found that one bulb showed the typical black sclerotia at the neck end. The fungus mycelium must have been present in the bulb during fumigation. Cultures confirmed the macroscopical observation. Four out of six cultures, made from different places among the onions, showed that, in this case at least, the gas was not effective against the fungus. This lot of onions was kept in storage until April 2, 1914, when they were discarded. Slightly over 10 per ct. of the bulbs showed the neck rot at that time. In this connection the experiments of Masee (1913) are of interest. This investigator experimented with the *Botrytis* causing the lily disease and found that pieces of lily stem containing the hibernating mycelium, even when soaked for twenty-four hours in hydrogen peroxid and later cultured, showed abundant growth and fruiting of the fungus.

In another experiment twelve apparently sound onions were placed in the fumigation chamber and treated for twenty-four hours with twice the amount prescribed in the Maine formula. After twenty-four hours the injured areas on the bulbs showed as raised blisters and the scales had a greenish tint. After being in storage forty days one of the twelve bulbs showed the neck-rot fungus breaking thru the outer scales. Four months later two of the bulbs were infected, showing that double strength gas treatment was not effective in controlling the fungus.

In a third series of experiments, both single and double strength gas caused conspicuous injury to the freshly exposed scales. When using the gas treatment upon either the colored or the white varieties of onions no injury occurred to the scales which were completely enclosed by a dry skin.

On October 6, 1913, a bushel crate of yellow onions was placed in the chamber and treated with the gas for twenty-four hours, after which it was removed and placed in a potato cellar along with another crate of onions from the same lot which was not fumigated. The storage conditions were perhaps typical of the average onion storage house in New York State. These were left undisturbed until April 6, 1914, or six months later, when they were removed and examined. Both the fumigated and unfumigated

stock showed a large amount (about 65 per ct.) of neck rot. Many of the bulbs showed the sclerotia at both ends, and many of the bulbs had sprouted and the fungus had reduced the sprouts to a mass of soft, brownish tissue.

Conclusions.—From these and similar experiments the writer is forced to conclude that fumigation of onions with formaldehyde gas against the neck rot is ineffective, mainly because it does not reach the mycelium in the bulbs. Furthermore, the average onion storage house is not tight enough to retain the fumes. The crates of onions usually contain a considerable amount of dry skins which prevents the penetration of the gas.

METHODS OF CONTROL.

From the data and experimental evidence at hand it is evident that the control of this disease lies mainly along the line of careful cultural measures and storage house control. Since it is evident that the greater part of the neck and bulb infection takes place late in the season, especially when the crop is curing, every precaution should be exercised to have the crop mature and die early. Late maturity may be prevented by avoiding the very late application of fertilizers and manures, especially fertilizers of an incorrect formula, and also by careful selection of the seed stock, being careful to avoid the types of onions with a thick neck. Air drainage has been found to have an indirect effect on the amount of neck rot and also an important bearing upon the prevention of onion mildew. The growing of onions in fields surrounded by hills and dense woods should be avoided when possible. Removing the tall weeds along ditches, or still better, by replacing the ditches with covered tile drains has been found to materially lessen the amount of neck rot, mainly by facilitating better drying of the crop. However, it is often necessary to provide wind-breaks of arbor vitae or picket fences along one side of the field to prevent wind injury to the crop.

Care should be exercised in handling the crop since infection occurs thru bruises, shovel, fork and topper wounds. As far as possible prevent bruising the stock while running it over the topping machine. The onions should be topped fairly short — 1 to 2 inches. The removal of excess soil, screenings, and tops by screening in the field will facilitate drying in crates. After a thoro drying

in the windrows the crates of onions should be kept in the cribs and drying sheds as long as possible. The bulbs should be stored in slatted crates of standard size. The use of bags or bins cannot be too strongly condemned. The full crates should be stacked with 1 x 3 inch binders between them, to facilitate ventilation. Onions stored in large, loose piles undergo a sweating process which aggravates the neck rot. The regulation of the onion storage house is most important. The neck rot cannot be controlled in storage unless good air circulation is provided by means of ventilators at top and bottom which can be opened and closed at will to control the temperature. The temperature of the storage house should be carried as low as possible without freezing the bulbs. A temperature just above 0° C., or 34°-36° F., is desirable. After the onions are thoroly chilled the storage should be kept closed to hold the temperature down. The storage house should not be opened for any considerable length of time especially during warm, damp, rainy or foggy days. If this is done the damp outside air will rush in and the entire stock will become covered with moisture and severe bulb rotting will follow. A relative humidity of 65-70° is very detrimental to keeping quality. Artificial heat, when needed, can best be supplied by stoves, or, at least, by dry heat. Steam heating is conducive to considerable rot since the steam pipes when not in use in cold weather become covered with frost and then, if steam is passed thru them, the humidity of the house is raised to a high degree.

Storage houses with a low, flat roof and poor top and bottom ventilation are to be avoided for onion storage. They should be constructed double thruout, using a good quality of lining for the walls. Concrete houses must have double walls with a dead-air space and must be sheeted up on the inside. The roof should have a steep pitch with large ventilators at the extreme top. The floors should be of heavy material with spaces of about one-half inch between each narrow piece of flooring. This is especially essential when onions are stored below the first floor.

The old, rotting onions should not be used as a fertilizer on the onion fields since it has been proved that they are a means of carrying the fungus to the fields and to the growing crop thru the mycelium and the sclerotia. Piles of decaying bulbs should not be permitted to remain in the vicinity of onion seed fields.

Especial attention should be given to the selection of sound, healthy seed or "mother bulbs" for the seed crop, since a few diseased bulbs may serve as a dangerous source of infection for the entire seed crop if the season is a wet one.

The treatment of the seed used for sowing the market crop by soaking it for 20 to 30 minutes in a solution made by adding 1 ounce of formalin to 2 to 3 gallons of water is being used by a number of onion growers. This is used as a preventive of onion smut and also as a precautionary measure against onion blight. Altho spores of the neck-rot fungus have been found on the seed, the possibilities of seed treatment for this disease have not been fully worked out experimentally. Seed treatment appears to be a safe precautionary measure. The light seed floating on the surface of the disinfecting solution may be skimmed off.

Spraying the crop with 5-5-50 bordeaux mixture or resin bordeaux, beginning early in July, and making 3 or 4 applications, has been found to be of some value in preventing neck rot, but extensive data on the value of spraying are not at hand. It has been practically demonstrated that the prevention of onion blight has a marked influence upon the amount of neck rot which may follow when poorly cured, soft, blighted stock is placed in storage.

Fumigation of yellow and red varieties of onions with gas either before or after the bulbs have been placed in storage cannot be recommended as a precautionary measure. In the first place onion storage houses are, as a rule, not tight enough to retain the fumes; furthermore, it has been found that the mycelium of the fungus is already within the necks of the bulbs before they go to the storage house. Any disinfectant which would kill the fungus in such cases would necessarily destroy the onions.

BIBLIOGRAPHY.

Aderhold, Rudolf.

1896 Die Fusicladien unserer Obstbäume. *Landw. Jahrb.*, 25:875-914.

Allen, C. E.

1901 On the origin and nature of the middle lamella. *Bot. Gaz.*, 32:1-34.

Ames, Adeline.

1915 The temperature relations of some fungi causing storage rots. *Phytopathology*, 5:11-19.

de Bary, A.

1863 Recherches sur le développement de quelques champignons parasites. *Ann. Sci. Nat. Bot.*, IV. 20:5-148.

1866 Neue Untersuchung ueber Uredineen II. Monatsber. d. Akad. d. Wiss. z. Berlin, p. 205.

-
- 1884 Morphologie und Physiologie der Pilze, Flechten, und Myxomyceten. Handbuch der physiologischen Botanik., 2:212-219. Leipzig.
-
- 1886 Über einige Sclerotien und Sclerotienkrankheiten. *Bot. Zeit.*, 44:377-387, 393-404, 409-426, 433-441, 449-461, 465-474.
-
- 1887 Comparative Morphology and Biology of the Fungi, etc., Eng. Ed. p. 45.
- Beauverie, J., and Guilliermond, A.
- 1903 Etude sur la structure du *Botrytis cinerea*. *Centbl. Bakt. (etc.) Abt. 2.*, 10:275-281.
- Behrens, J.
- 1898 Beiträge zur Kenntnis der Obstfäulnis. *Centbl. Bakt. (etc.) Abt. 2.*, 4:514-522, 547-553, 577-585, 635-644, 700-706, 739-746, 770-777.
- Bigelow, W. D., Gore, H. C., and Howard, B. J.
- 1905 Studies on apples. U. S. Dept. Agr., Bur. Chem. Bul., 94:1-99.
- Blackman, V. H., and Welsford, E. J.
- 1916 Studies in the physiology of parasitism II. Infection by *Botrytis cinerea*. *Ann. Bot.*, 30:389-398.
- Blair, Fred. J.
- 1916 Development and localization of truck crops in the United States. Separate from Yearbook of the Department of Agriculture, 1916. No. 702, p. 12.
- Bourquelot, Em.
- 1899 Sur la pectase. *Jour. Pharm. et Chim.*, 9:563-568; 10:5.
- Bourquelot, Em., and Herissey, H.
- 1898 Sur l'existence, dans l'orge germée, d'un ferment soluble agissant sur la pectine. *Compt. Rend. Acad. Sci. (Paris)*, 127:191-194.
-
- 1898 De l'action des ferments soluble sur les produits pectiques de la racine de gentiane. *Jour. Pharm. et Chim.* 8:145-150.
- Brooks, Charles, and Cooley, J. S.
- 1917 Temperature relations of apple-rot fungi. *Jour. Agr. Research*, 8:139-164.
- Brooks, F. T.
- 1907 Notes on the parasitism of *Botrytis*. *Proc. Cambridge Phil. Soc.*, 14:3, p. 298.
-
- 1908 Observations on the biology of *Botrytis cinerea*. *Ann. Bot.*, 22:479-484.
- Brown, Wm.
- 1915 Studies on the physiology of parasitism. I. The action of *Botrytis cinerea*. *Ann. Bot.*, 29:313-348.
-
- 1916 Studies in the physiology of parasitism. III. On the relation between the "infection drop" and the underlying host tissue. *Ann. Bot.*, 30:399-406.
- Bruck, W. Friedrich.
- 1907 Pflanzenkrankheiten. p. 83. Leipzig.
- Bruschi, Diana.
- 1912 Attività enzimatiche di alcuni funghi parassiti di frutti. *Atti. R. Accad. Lincei, Rend. Cl. Sci. Fis., Mat. e. Nat.*, S. 5, V. 21, sem. 1, fasc. 3, pp. 225-230; fasc. 4, pp. 298-304.
- Clinton, G. P.
- 1903 Rpt. of Station Botanist. 27th Ann. Rpt. Conn. Agr. Expt. Sta., 1903:334-336.
-
- 1904 Rpt. of Station Botanist. 28th Ann. Rpt. Conn. Agr. Expt. Sta., 1904:321-322.

-
- 1914 Rpt. of Station Botanist. 38th Ann. Rpt. Conn. Agr. Expt. Sta., 1914:4.
- Cook, M. T., and Schwarze, C. A.**
1913 A Botrytis disease of dahlias. *Phytopathology*, 3:171-175.
- Cooley, J. S.**
1914 A study of the physiological relations of *Sclerotinia cinerea* (Bon.) Schröter. *Ann. Mo. Bot. Gard.*, 1:291-327
- Coons, G. H.**
1916 Factors involved in the growth and the pycnidium formation of *Plenodomus fuscomaculans*. *Jour. Agr. Research*, 5:713-769
- Cross, C. F., and Bevan, E. J.**
1895 An outline of the chemistry of the structural elements of plants. London.
- Delacroix, G., and Maublanc, A.**
1909 Maladies parasitaires des plantes cultivées. p. 401. Paris.
- Dox, A. W.**
1910 The intracellular enzymes of *Penicillium* and *Aspergillus*. U. S. Dept. Agr. Bur. An. Ind. Bul., 120:1-70.
- Emmerling, O.**
1903 Oxalsäurebildung durch Schimmelpilze. *Centbl. Bakt.* (etc.) Abt. 2., 10:273-275.
- Euler, Hans.**
1912 General chemistry of the enzymes. New York. 1-319.
- Frank, A. B.**
1880 Die Krankheiten der Pflanzen. 540-542. Breslau.
-
- 1896 Die Krankheiten der Pflanzen. 2:503-505. Breslau.
- Franceschelli, Donato.**
1915 Untersuchungen über die Enzyme in Mycelien des auf stickstoffrein Stärekuchen gezüchteten *Penicillium glaucum*. *Centbl. Bakt.* (etc.) Abt. 2., 43:305-322.
- Fremy, Edmond.**
1840 Premiers essais sur la maturation des fruits. Recherches sur la pectine et l'acide pectique. *Jour. Pharm. et Chim.*, 26:368-393.
-
- 1848 Memoirs sur la maturation des fruits. *Ann. Chim. et Phys.*, 3:24:1-58.
- Green, J. Reynolds.**
1901 The soluble ferments and fermentation. Cambridge. Second Ed., 298-300.
- Haas, Paul, and Hill, T. G.**
1913 An introduction to the chemistry of plant products. pp. 338-339. London and New York.
- Halsted, B. D.**
1890 Fungous diseases of various crops. Rpt. N. J. Agr. Expt. Sta., 3:352.
- Hanzawa, Jun.**
1915 *Fusarium cepae*, ein neuer Zwiebelpilz Japans, sowie einige andere Pilze an Zwiebelpflanzen. *Mycol. Centbl.*, 5:11-12.
- Harter, L. L.**
1916 Storage rots of economic aroids. *Jour. Agr. Research*, 6:549-571.
- Hoffmann, Hermann.**
1860 Untersuchungen über die Keimung der Pilzsporen. *Jahrb. Wiss. Bot.* (Pringsheim), 2:267-297.
- Humbert, J. G.**
1916 The neck rot of white onions. Mo. Bul. Ohio Agr. Expt. Sta., 1:176-180.
- Istvánfi, Gy. de.**
1905 Etudes microbiologiques et mycologiques sur le rot gris de la vigne-*Botrytis cinerea* ou *Sclerotinia Fuckeliana*. Ann. Inst. Cent. Ampéol. Roy. Hongrois.

Iterson, C. van, Jr.

- 1904 Die Zersetzung von Cellulose durch aërobe Mikroorganismen. *Centbl. Bakt. (etc.)* Abt. 2, 11:689-698.

Jackson, H. S.

- 1914 Second biennial crop pest and horticultural report. Oregon Agr. Expt. Sta., 1913-1914:269-270.

Jarvis, C. D.

- 1909 Stem rot of onion. Conn. (Storrs) Agr. Expt. Sta., Bul. 56:258.

Jensen, C. N.

- 1912 Fungous flora of the soil. Cornell Agr. Expt. Sta., Bul. 385:415-501.

Jones, L. R.

- 1909 Pectinase, the cytolytic enzym produced by *Bacillus carotovorus* and certain other soft-rot organisms. N. Y. Agr. Expt. Sta. Tech. Bul. 11:291-368. Ibid. Vt. Agr. Expt. Sta. Bul., 147:243-360. 1910.

Jones, L. R., Giddings, N. J., and Lutman, B. F.

- 1912 Investigations of the potato fungus *Phytophthora infestans*. Vt. Agr. Expt. Sta. Bul., 168:1-100; also, U. S. Dept. Agr. Bur. Plant Indus. Bul. 245:1-100.

Kean, A. L.

- 1890 On the nature of certain plant diseases. *Bot. Gaz.*, 15:171-174.

Kissling, E.

- 1889 Zur Biologie der *Botrytis cinerea*. *Hedwigia*, 28:227.

Kühn, J.

- 1858 Die Krankheiten der Kulturgewächse, ihre Ursachen und ihre Verhütung. pp. 1-312. Berlin.

Lendner, Alfred.

- 1896 Des influences combinées de la lumière et du substratum sur le développement des champignons. *Ann. Sci. Nat. Bot.* 8, 3:1-64.

Lutz, Otto.

- 1909 Ueber den Einfluss gebrauchter Nährlösungen auf die Keimung und Entwicklung einiger Schimmelpilze. *Ann. Mycol.*, 7:91-133.

Mangin, L.

- 1892 Propriétés et réactions des composés pectiques. *Jour. Bot. [Paris]*, 6:206-212, 235-244, 363-368.

- 1893 Recherches sur les composés pectiques. *Jour. Bot. [Paris]*, 7:37-47, 121-131, 325-343.

Massee, Geo.

- 1894 An onion disease. *Gard. Chron.*, 3:160.

- 1903 A textbook of plant diseases. p. 156. London.

- 1914 Diseases of cultivated plants and trees. p. 264. New York.

Massee, Ivy.

- 1913 *Jour. Board of Agr. and Fisheries*, 20:9. London.

Michaelis, Leonor.

- 1910 Methoden zur Darstellung von Fermenten. Handbuch der Biochemischen Arbeitsmethoden. 3 (Erste Hälfte): 13.

Miyoshi, Manabu.

- 1894 Ueber Chemotropismus der Pilze. *Bot. Ztg.*, 52:1-28.

Molisch, Hans.

- 1913 Mikrochemie der Pflanze. Jena.

Morse, W. J.

- 1907 Potato diseases in 1907. Me. Agr. Expt. Sta. Bul. 149.

Nordhausen, M.

- 1899 Beiträge zur Biologie parasitärer Pilze. *Jahrb. Wiss. Bot.*, 33:1-46.

Novy, F. G.

- 1899 Laboratory work in bacteriology. p. 513. Ann Arbor.

- O'Brien, A.
1902 The comparative resistance to high temperatures of the spores and mycelium of certain fungi. *Bul. Torrey Bot. Club*, 29: 170-172.
- Oudemans, C. A. J. A.
1884 *Nederl. Kruidk. Arch.* 2, 4: 260.
- Peltier, G. L.
1912 A consideration of the physiology and life history of a parasitic *Botrytis* on peppers and lettuce. *Mo. Bot. Gard. Ann. Rpt.*, 23: 41-74.
- Pfeffer, W. F. P.
1900 The physiology of plants. 2nd ed., V. 1: 275. (Transl. by Ewart.) London.
- Price, S. R.
1911 Peculiar spore forms of *Botrytis*. *New Phytologist*, 10: 255-259.
- Prillieux, Ed.
1897 *Maladies des plantes agricoles.* 2: 431-433. Paris.
- Reed, H. S.
1912 The enzym activities involved in certain fruit diseases. *Ann. Rpt. Va. Agr. Expt. Sta.*, 1911-1912: 51-78.
- Reidemeister, W.
1909 Die Bedingungen der Sklerotien- und Sklerotienringbildung von *Botrytis cinerea* auf künstlichen Nährböden. *Ann. Mycol.*, 7: 19-44.
- Ridgway, Robert.
1912 Color standards and color nomenclature. pl. 46. Washington.
- Ruhland, W.
1908 Beitrag zur Kenntnis des sogenannten "Vermehrungspilzes." *K. biol. Anst. F. Land- u. Forstw.*, Arb. 6: 71-76.
- Schellenberg, H. C.
1908 Untersuchungen über das Verhalten einiger Pilze gegen Hemizellulosen. *Flora*, 98: 257-308.
- Schröter, J.
1879 Weisse Heidelbeeren. Eine Pilzkrankheiten der Beeren von *Vaccinium myrtillus* L. *Hedwigia*, 18: 182.
- Selby, A. D.
1910 A brief handbook of the diseases of cultivated plants in Ohio. *Ohio Agr. Expt. Sta. Bul.*, 214: 413.
- Smith, R. E.
1900 *Botrytis* and *Sclerotinia*: Their relation to certain plant diseases and to each other. *Bot. Gaz.*, 29: 369-407.
1902 The parasitism of *Botrytis cinerea*. *Bot. Gaz.*, 33: 421-436.
- Sorauer, P.
1876 Das Verschimmeln der Speisezwiebeln. *Oesterr. Landw. Wchnbl.* p. 147.
1886 Sclerotienkrankheiten (das Verschimmeln) der Speisezwiebeln. *Handbuch der Pflanzenkrankheiten*, Aufl. 2, 2: 294-297.
Lindau, G., and Reh, L.
1908 *Handbuch der Pflanzenkrankheiten*, Aufl. 3, 2: 301-302.
- Spieckermann, A.
1902 Beitrag zur Kenntnis der bakteriellen Wundfäulnis der Kulturpflanzen. *Landw. Jahrb.*, 31: 155-178.
- Stevens, N. E.
1916 Pathological histology of strawberries affected by species of *Botrytis* and *Rhizopus*. *Jour. Agr. Research*, 6: 361-367.
- Tunmann, Otto
1913 *Pflanzenmikrochemie.* Berlin.
- Valleau, W. D.
1915 Varietal resistance of plums to brown rot. *Jour. Agr. Research*, 5: 365-396.

- Voges, E.
1910 Die Bekämpfung des Fusicladium. *Ztschr. Pflanzenkrank.*, 20: 385-393.
- Voglino, P.
1903 Sul parassitismo e lo sviluppo dello *Sclerotium cepivorum* Berk., nell, *Allium sativum* L. *Staz. Sper. Agr. Ital.*, 36: 2. p. 89-106.
- Wakker, J. H.
1883 Verläufige Mittheilungen über Hyacinthenkrankheiten. *Bot. Centbl.*, 14: 315.
- Ward, H. M.
1888 A lily-disease. *Ann. Bot.*, 2: 319-382.
- Wehmer, C.
1891 Entstehung und physiological Bedeutung der Oxalsäure im Stoffwechsel einiger Pilze. *Bot. Ztg.*, 49: 233-246, 249-257, 271-280, 289-298, 305-313, 321-332, 337-346, 353-363, 369-374, 385-396, 401-407, 417-428, 433-439, 449-456, 465-478, 511-518, 531-539, 547-554, 563-569, 579-584, 596-602, 611-620, 630-638.
- Zimmermann, A.
1893 Botanical Microtechnique. New York.
- Zschokke, A.
1897 Ueber den Bau der Haut und die Ursachen der verschiedenen Haltbarkeit unserer Kernobstfrüchte. *Landw. Jahrb. Schweiz.*, 11: 153-196.

